

# CONTRIBUTIONS TO EMBRYOLOGY

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A MEMORIAL TO  
FRANKLIN PAINE MALL



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## FOREWORD.

The papers included in this volume have been contributed as a memorial by present and former members of the staff of the late Professor Franklin Paine Mall, in recognition of his inspiring leadership and in response to the strong feeling of affection with which they had come to regard him. A volume of this nature had been under consideration, to commemorate the approaching twenty-fifth anniversary of his occupancy of the chair of anatomy in the Johns Hopkins University. His untimely death, however, just at the close of a quarter century of remarkable productivity, interfered with the project as originally planned and left it possible to offer only a belated tribute in the form of the present volume.

BALTIMORE, *August 1, 1919.*

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# LIST OF CONTRIBUTIONS.

	Page.
BARDEEN, C. R. The height-weight index of build in relation to linear and volumetric proportions and surface area of the body during post-natal development. (11 charts, 2 text-figures.) No. 46...	483-554
BEAN, ROBERT B. Notes on the post-natal growth of the heart, kidneys, liver, and spleen in man. (8 text-figures.) No. 37.....	263-284
CLARK, ELIOT R., and ELEANOR L. CLARK. On the origin and early development of the lymphatic system of the chick. (7 plates, 15 text-figures.) No. 45.....	447-482
CORNER, GEORGE W. On the widespread occurrence of reticular fibrils produced by capillary epithelium. (7 plates.) No. 29.....	85-93
DUESBERG, J. Cytoplasmic structures in the seminal epithelium of the opossum. (2 plates, 5 text-figures.) No. 28.....	47-84
ESSICK, CHARLES R. Formation of macrophages by the cells lining the subarachnoid cavity in response to the stimulus of particulate matter. (1 plate.) No. 42.....	377-388
JENKINS, GEORGE B. A study of the superior olive. (2 plates, 1 text-figure.) No. 33.....	157-172
LEWIS, MARGARET R. Muscular contraction in tissue cultures. (2 plates, 6 text-figures.) No. 35.....	191-212
LEWIS, WARREN H. The cartilaginous skull of a human embryo 21 millimeters in length. (5 plates.) No. 39.....	299-324
MACKLIN, CHARLES C. The development and function of macrophages in the repair of experimental bone-wounds in rats vitally stained with trypan-blue. (4 plates.) No. 27.....	1-46
MEYER, ARTHUR W. Hydatiform degeneration in tubal and uterine pregnancy. (6 plates.) No. 40.....	325-364
MILLER, WILLIAM SNOW. A morphological study of the tracheal and bronchial cartilages. (2 plates, 11 text-figures.) No. 38.....	285-298
MYERS, BURTON D. A study of the development of certain features of the cerebellum. (6 figures.) No. 41.....	365-375
RETZER, ROBERT. The sino-ventricular bundle: A functional interpretation of morphological findings. (1 plate.) No. 32.....	143-156
SABIN, FLORENCE R. Studies on the origin of blood-vessels and of red blood-corpuscles as seen in the living blastoderm of chicks during the second day of incubation. (6 plates, 1 text-figure.) No. 36..	213-262
SCHULTZ, ADOLPH H. The development of the external nose in whites and negroes. (1 plate, 7 figures.) No. 34.....	173-190
STREETER, GEORGE L. A human embryo (Mateer) of the presomite period. (7 plates, 4 text-figures.) No. 43.....	389-424
VAN DER STRICHT, O. The arrangement and structure of sustentacular cells and hair-cells in the developing organ of Corti. (4 plates.) No. 31	109-142
WEED, LEWIS H. The experimental production of an internal hydrocephalus. (2 plates.) No. 44.....	425-446
WHEELER, THEODORA. Variability in the spinal column as regards incomplete closure of the dorsal vertebral laminae (rudimentary spina bifida). (1 plate.) No. 30.....	95-107

A paper was also submitted by Professor Herbert M. Evans but too late for incorporation in this Memorial; it will appear in the next volume of Contributions to Embryology.





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CONTRIBUTIONS TO EMBRYOLOGY, No. 27.

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THE DEVELOPMENT AND FUNCTION OF MACROPHAGES IN THE  
REPAIR OF EXPERIMENTAL BONE-WOUNDS IN RATS  
VITALLY STAINED WITH TRYPAN-BLUE.

BY CHARLES CLIFFORD MACKLIN,  
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With four plates.

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## CONTENTS.

	PAGE.
Introduction.....	3
Material and method.....	4
Observations.....	6
Discussion.....	28
Extraosseous macrophages.....	29
Development.....	31
Mobilization.....	33
Activity.....	34
Decline.....	35
Intraosseous macrophages.....	36
Development.....	36
Activity.....	37
Decline.....	40
Comparison of extraosseous and intraosseous macrophages.....	41
Conclusions.....	43
Bibliography.....	44
Description of figures.....	45

# THE DEVELOPMENT AND FUNCTION OF MACROPHAGES IN THE REPAIR OF EXPERIMENTAL BONE-WOUNDS IN RATS VITALLY STAINED WITH TRYPAN-BLUE.

BY CHARLES CLIFFORD MACKLIN.

## INTRODUCTION.

The *vital-staining route* as an approach to the problem of bone-repair came as a natural consequence of the recent work of Shipley and Macklin (1916<sup>1,2</sup>) on osteogenesis. By subjecting very young, growing animals to trypan-blue, one of the azo-dyes belonging to the benzidine series of colors, these investigators were able to show that the regions of active bone-growth took a more intense stain than the remainder of the bone; and, furthermore, that the heightened coloration was largely referable to the presence in these areas of innumerable phagocytic cells, within whose cytoplasm the dyestuff was stored in multitudinous tiny segregations known as "dye-granules."

These phagocytes were identified as the reticulo-endothelial cells of the young bone-marrow. Their reaction to the dyestuffs of the benzidine group is the same as that of the host of cells found throughout the body, which have been extensively studied by different authors, and to which various names have been given, such as "pyrrhol-cells" (Goldmann, 1909), "elasmatoocytes" (Ranvier, 1899-1900), "resting-wandering cells" (Maximow, 1906), etc. Recently Evans (1915) has employed Metschnikoff's term "macrophage" to cover this entire group of phagocytic cells which are united by a uniform functional response to these colloidal dyestuffs, and it is now well recognized that the term "macrophage" is a physiological designation, including within its compass very diverse morphological elements. This similar staining reaction, indeed, is but an expression of the phagocytic potentiality which these cells hold in common (Evans and Schulemann, 1914) and which manifests itself during their every-day existence in the ingestion and storage of certain elements of the surrounding tissue-fluids.

In consideration of the vigorous phagocytic properties attributed to these cells, and also of their being present in large numbers where temporary bone and cartilage were being absorbed, it seemed evident to Shipley and Macklin (1916<sup>2</sup>) that they were a very important factor in active osseous development and that their peculiar rôle under these circumstances was played in connection with the resorption of the provisional cartilage and bone.

Now bone-resorption is an active process in the later stages of bone-repair, for it is well known that the excess of provisional callus which is built up following a bone-injury, such as a fracture, is gradually removed. Since the resorption of this provisional callus is quite similar to that of provisional new bone it was decided to investigate the vitally stained cells in the callus of healing bone-wounds and to compare the findings with those in young, growing bone.

In the earlier stages of the reparative process following bone-wounds, too, there is a great deal of débris to be eliminated, such as injured bone, blood-clot, damaged muscle, and other devitalized tissue, and it is plain from the writings of other workers that the potentialities of the macrophages eminently fit them for the performance of this duty. That they play a part in the healing of wounds of soft tissues, such as skin, kidney, and liver, may be inferred from the work of Goldmann (1912), who demonstrated by the aid of vital-dyes that they were increased in the regions where repair was proceeding. Indeed, the evidence of numerous investigators points to the macrophages being concerned in all inflammatory conditions. Maximow (1902, 1909<sup>2</sup>), notably, has made a special study of these cells in inflammatory areas, where he finds them increased in number and size, and speaks of them as "polyblasts"; and Tschasehin (1913) has recorded similar findings.

The problem of the healing of wounds of bone, therefore, seemed to offer a particularly favorable field for the application of the vital-staining method, for it was expected that in the early stages of bone-repair, where damaged soft parts must be cleared away, as well as in the later stages, where provisional bone has to be eroded, the trypanophil cells—*i. e.*, the macrophages—in the pursuance of their physiological vocation as phagocytes, would become locally very numerous and would show hypertrophy and intensified phagocytic power. These expectations, as will be seen, were realized, and the following pages are devoted to the discussion of the gross and microscopic appearances presented in the progressive stages of healing of fractures and trephine wounds in rats in whose tissues the macrophages were made visible by the introduction of the dyestuff (trypan-blue) into the circulating fluids shortly before death.

### MATERIAL AND METHOD.

For the most part, the experiments were carried out on the albino rat, though the black and crossed breeds were also used. The material was collected at the admirable rat colony of the Wistar Institute of Anatomy and Biology, Philadelphia,\* and this insures that the rats were all perfectly healthy and were kept under the most favorable conditions during the time of experimentation. A complete series of stages was secured, covering the entire period of repair.

The operations were conducted as follows: The animal having been anesthetized with ether, the top of the head was carefully shaved and sterilized; a median incision was then made and the skin reflected over the parietal areas. The parietal bone having been laid bare, a small trephine of 5 mm. diameter was used to perforate it. In some cases the piece was removed altogether; in others it was replaced, sometimes upside down; and in still other cases a piece of living or dead bone from another rat, or even dead bone from an animal of a different species, was inserted. As a rule two areas were trephined, one in each parietal bone. The wounds were

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\*I wish to thank Drs. Greenman and Donaldson, of the Wistar Institute, for laboratory facilities and access to the rat colony; also Dr. McClung, of the University of Pennsylvania, for placing his laboratory at my disposal for the operations. Finally, I wish to express to Miss Madge DeG. Thurlow my most cordial thanks for the invaluable assistance rendered by her in carrying on this investigation.



carefully sutured with sterile silk, and the most rigid asepsis was observed throughout. In the same animals, before recovery from the anesthetic, the tibia and femur of the left hind leg and a few of the ribs on the left side were fractured. No attempt was made to splint the bones, so that healing took place under the same conditions as in natural life.

There were slight variations from this general type experiment; in some animals only trephining was done and in others only fracturing. In all, 20 animals were used, which furnished 16 trephined skulls, 15 fractured tibiae, 15 fractured femora, and 12 sets of fractured ribs. In table 1 a full description of the material is given.

TABLE 1.—*Material.*

Day of repair.	Animal No.	Age at death (months).	Specimens examined.							
			Skull.		Rib.		Long bones.			
			Cleared gross.	Sec-tions.	Cleared gross.	Sec-tions.	Tibia.		Femur.	
							Cleared gross.	Sec-tions.	Cleared gross.	Sec-tions.
2.....	S 18-1	3	sc		sc		sc	sc	sc	
3.....	S 11-1	2½	sc	sc	sc		sc	sc	sc	sc
5.....	S 12-1	3		sc	sc		sc	sc	sc	sc
6.....	S 11-2	2½	sc	sc	sc		sc	sc	sc	sc
6.....	S 13-1	3	sc	sc	sc		sc	sc	sc	sc
9.....	S 17-2	3	sc		sc		sc		sc	
10.....	S 5-1	3	sc	sc						
10.....	S 6-1	3					sc	sc		
10.....	S 6-2	3							sc	sc
12.....	S 5-2	7	sc	sc					sc	sc
13.....	S 11-3	3	sc		sc		sc		sc	
15.....	S 5-3	7½		sc			sc	sc		
20.....	S 12-2	3½	sc		sc		sc	sc	sc	
20.....	S 13-2	3	sc		sc		sc	sc	sc	
30.....	S 11-4	3½	sc		sc		sc	sc	sc	
51.....	S 17-1	4	sc		sc		sc	sc	sc	
58.....	S 15-1	14	sc							
59.....	S 12-3	4½	sc				sc	sc	sc	
60.....	S 11-5	4½	sc	sc	sc		sc	sc	sc	
71.....	S 5-5	17	sc							
16 stages	20 animals		16 skulls, 8 sec-tioned.		12 ribs, none sec-tioned.		15 tibiae, 13 sec-tioned.		15 femora, 6 sec-tioned.	

sc = specimen examined.

The animals were killed at various periods during the repair process, the specimens ranging from the second to seventy-first day of healing, as shown in table 1. The vital-staining technique was the same as that used by Shipley and Macklin (1916<sup>1,2</sup>) in their work on developing bone. Shortly before the time selected for killing, trypan-blue was administered intraperitoneally in the form of a sterile 1 per cent aqueous solution. As a rule, the dyestuff was given 48 hours before killing and repeated after 24 hours, so that the macrophages were exposed to its action for 2 days. Occasionally the period of exposure was as short as 1 or as long as 3 days. No ill effects followed the exhibition of the dye.

At the time selected the animals were anesthetized and bled, the required tissues being at once dissected out and fixed in 10 per cent formalin neutralized with magnesium carbonate. Care was taken to leave the soft parts surrounding the bones undisturbed. After fixing for 24 hours and washing in running tap-water for the same length of time the tissues were passed through a graded series of alcohols ending with two changes of absolute. They were then cleared in benzine and oil of wintergreen, after the method of Spalteholz (1914), and examined. Of the cleared specimens the ribs and skulls were most satisfactory, for it was impossible to make the larger bones transparent because of their greater density and thickness.

Certain of the skulls and long bones were then decalcified, embedded, and sectioned (table 1), so that the various stages could be studied microscopically. A few were cleared after having been decalcified; some of these also were afterwards sectioned. The most valuable sections were those simply cleared and mounted with no stain other than the trypan-blue and those lightly counterstained with carmine, which afforded a satisfactory contrast with the blue. Hematoxylin and eosin for cellular detail were used, and also (in special cases) methyl-green and safranin.

### OBSERVATIONS.

In the description of the gross and microscopic findings in the vitally stained healing wounds of bone, the different stages, as shown by a study of cleared gross specimens of ribs and skulls and of cleared and stained sections, will be taken up in order. Later on, the various aspects of vital staining will be analyzed, consolidated, and finally reduced to a summary. But before proceeding to a portrayal of the changes in the vital staining of wounded bone and the tissues immediately surrounding the site of the wound it will be well to describe briefly the appearance of the vitally stained normal bone and its tissue environment.

A good example of this is seen in figure 1, drawn with the aid of the binocular microscope from a cleared rib taken from a rat vitally stained for 2 days with trypan-blue. It is intended as a control of the stages afterward to be described.

The clear shaft of the bone (B) is easily seen surrounded by the periosteum (P) and inclosing a well-marked medullary canal (MC). To the left the intercostal vessels and nerve (N) are faintly sketched in. The fasciculi of intercostal muscle (MUS) are suggested by the intercrossing lines.

It will be noted at once that black dots appear throughout the drawing. These are seen as blue granules in the original specimen and represent individual cells which have phagocytized and stored the dyestuff. These cells are more concentrated in the medullary cavity, the periosteum, and in the connective tissue around the bone. In the intermuscular septa they are conspicuous as granular streaks. These cells are macrophages and their presence in the regions described, as is well known, is normal. Goldmann (1909), for instance, has recorded similar findings, as in his Taf. XI, No. 2, where he shows the histological appearance of the vitally stained interfibrillar cells in the muscle of the tongue.

There are, then, both within the shaft of the bone and in the tissues of its immediate environment, under normal conditions, appreciable numbers of macrophages. In the next section it will be found that, following bone injury, their numbers are locally very much increased.

#### SECOND-DAY STAGE.

The earliest stage of bone repair examined was that at the end of 2 days (animal S 18-1).

On inspection of the *cleared ribs* with the binocular microscope the most obvious change, as compared with the control, is the marked blue staining of the broken ends of bone and of the material surrounding them. The ends of the medullary canal are plugged with it. This staining is shown in black\* in figure 2. It is very dense and irregular in character and quite different from the granular type of staining presented in the control rib (fig. 1). The impression conveyed is that the tissue injured by the trauma, and the associated exudate, have absorbed the dye. There is as yet very little swelling at the site of the fracture, so that the contour of the bone, apart from the distortion of the break, is the same as that of the control.

Under the highest power of the binocular the blue granules, representing macrophages, are seen, as in the control, in the periosteum and marrow cavity of the bone, and in the surrounding connective tissue. At the site of the fracture, however, it is noted that they are more numerous than elsewhere and they have apparently been increased in this region. In the drawing (fig. 2) they appear as a small cloud of granules near the broken bone-ends and around the densely colored material. This granular appearance is a true intra-vital dyeing, and stands in sharp contrast to the diffuse and dense staining above described. The periosteum as yet shows no obvious changes, such as thickening.

In the *cleared skull*, as in the ribs, a dense and diffuse blue staining is seen in the bone fragments and in other dead tissue resulting from the operation. Macrophages are not definitely increased.

In the *cleared long bones* the tissues of the fracture-precinct are densely stained, but the specimen, on account of its thickness and opacity, is unsatisfactory for inspection of the macrophage tissue.

As would be expected from the cleared specimens, the uncounterstained *sections of long bone* (tibia) show areas near the broken ends where the misshapen and apparently injured tissue has taken a dense, irregular, and diffuse blue stain. Under higher powers such areas appear as shapeless blue masses of fragmented muscle-fibers, a little fibrous tissue, blood, and invading phagocytes, interspersed with clearer areas of exudate. Often individual muscle-fibers appear stained blue throughout. There is evidently much cell liquefaction going on.

Diffuse staining of this type is an indication of cell death (MacCurdy and

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\*All blue staining is represented diagrammatically in the illustrations by the denser black.

Evans, 1912; Evans and Schulemann, 1914), and hence it may be concluded that this diffusely stained material consists of devitalized protoplasm.\*

The sections also confirm the cleared preparations in revealing at the fracture-site an increase in the vitally stained phagocytes as compared with corresponding areas in control preparations. These blue cells are present among the degenerate tissue-remnants, extravasated blood, and exudate. Often large macrophages filled with blue granules appear in the blood-clot, and here the corpuscles have almost disappeared, showing that there is a direct relationship between the presence of the macrophages and the disappearance of the blood-cells. The same association is emphasized in the damaged muscle and other tissue.

In addition to the large, irregularly shaped cells, which we may identify as the clasmatocytes—the normal mononuclear tissue-resident phagocytes—which show the most distinct blue granulation, there are great numbers of smaller dye-containing cells. These are of various sizes, extending from a small, round, lymphocyte-like cell to the large polyblast. In the smaller cells the dyestuff is very slight, even absent, and as a rule it is increased in amount progressively in the larger sizes. It would seem that the larger cells are developed from the smaller and that the cytoplasm not only hypertrophies, but with this acquires the power of imbibing and storing the colloidal dyestuffs in granule form. The macrophages are always larger and more numerous where muscle and other tissue is evidently damaged. Later stages will show that there is always an excess of macrophage tissue where protoplasm is degenerate, as evidenced by its diffuse staining with trypan-blue and by its histological characters.

Polymorphonuclear leucocytes are often seen in the tissues of the fracture-area. No dye-granules were observed in them. Quite a number of fibroblasts were found; occasionally a few small grains of blue were noted in them, but they take the dyestuff very sparingly. There are, too, many tiny blood-vessels—in fact we have here to deal with granulation tissue.

On the second day the sections show the beginning of the *callus*. This is a fairly thick layer of basophilic cells lying along the original bone in the region of the injury. These cells are evidently young osteoblasts, and it is plain that there has been an extensive proliferation of them. Overlying this osteoblastic precallus is a layer of fibrous connective tissue continuous on either side with the periosteum.

In the precallus there are traces of new bone in the form of slender plates, lying along the original bone, and, in places, short delicate trabeculae arising from

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\*The trypan-blue staining which occurs in (a) devitalized tissue has several outstanding and distinctive characteristics. The blue mass is made up of what appears to be degenerate tissue, in which the cells are often misshapen and broken up, or it may be clumped together in a disorderly manner and mixed with exudate. The staining is dense and irregular and involves the entire cell, including the nucleus. It is simply due to the absorption of the dyestuff by the necrotic protoplasm. Its occurrence is thus good evidence of the presence of defunct tissue and exudate. Living protoplasm is able to protect itself by excluding or segregating the dye, except as described below in "c."

This typical behavior of necrotic or moribund tissue toward trypan-blue is to be sharply distinguished from the (b) true vital-staining, which is found in the macrophages. Here the dyestuff is housed within the cytoplasm in the form of numerous discrete droplets or granules. Often the dye is found in the fluid of a vacuole. The nucleus does not take the dye. Under low magnification, as with the binocular microscope, the entire cell is seen as a single blue granule, standing out in sharp contrast to its unstained surroundings. Such an appearance is represented in the drawings of the cleared ribs (figs. 1 to 5) and cleared skull (fig. 6).

A third type of trypan-blue staining (c), distinct from both of the above, may be referred to here. It is found in certain relatively inert living tissue (such as the elastic laminae of blood-vessels), in fibrous connective tissue (as tendons and ligaments), and in scar tissue. It is a rather pale, even coloration and is easily distinguishable from the true vital staining in the macrophages and from the staining of devitalized tissue.



these into the precallus. This material is directly continuous with the old bone. Ossification is so slight that it is difficult to find manifestations of it. Occasionally spaces, containing one or two osteoblasts, occur between the spicules of bone. Vessels have hardly begun to invade this precallus, a small capillary being found but rarely in its peripheral layer.

The principal features of the second-day stage, then, may be summed up as follows: (1) the presence of increased numbers of vitally stained macrophages in the tissues surrounding the fracture which have been damaged by the trauma, and in the exudate associated with this; (2) the diffuse blue staining of dead and damaged tissue; (3) the first indication of the callus.

#### THIRD-DAY STAGE. (S 11-1).

The next stage studied was that at the end of 3 days' repair. In the *cleared ribs* (fig. 3) it is at once seen that there has been a great change at the site of the fracture, which appears very much more intensely stained than at the earlier stage. As at the second day, the position of the approximated ends of the bone is marked by an opaque blue irregular mass, representing the diffusely stained areas of injured bone and soft parts; beyond this the outline of the bone-shaft may be easily followed.

But the most characteristic thing is the dense, cloud-like, blue sheath which surrounds the fracture-area and gives to it a swollen appearance. Upon examining the specimen critically with the binocular, the blue staining may largely be resolved into granules, each of which represents a macrophage. The appearance is as though the normal resident macrophages had become immensely multiplied in the preceding 24 hours, or as though great numbers of macrophages had invaded the area from other parts. Many of the cells occur in rows between the muscle strands, giving rise to the appearance of blue granular streaks.

Between this investment of macrophagic tissue and the bone there is a clearer area, and as this is followed in either direction along the bone it is seen to underlie a membrane which is continuous with the periosteum. No definite periosteum is found on the bone which it covers. It represents the young callus. Its vigorous proliferation around the ends of the bone has caused the swelling which has resulted in an outpushing of the macrophagic zone.

In the *long bones* an intense blue staining at the site of the bone-wound is extremely conspicuous in the fresh condition. As in the ribs and skull, it is due to the diffuse staining of injured tissue as well as to the macrophagic invasion. The *sections* of the long bones show a diffuse blue-staining of the broken ends (shown in black in fig. 7, B), and also a dense blue-staining of the exudate and loose débris, of which there is abundance. This dense staining is even more marked than on the second day.

These sections are very striking. A good example of a specimen field is seen in figure 7. This is a low-power photomicrograph from a tangential section through the fracture-area; in it myriads of blue cells (shown as black dots M) crowd about the broken bone and through the surrounding injured tissues and extravasated blood. There is a great deal of fragmented muscle and the blue macrophagic cells

surround the remnants of this, and even squeeze in between the fibers. Under the high power they swarm among the fibroblasts and polymorphonuclear leucocytes, around the broken bone-ends, in the open spaces—everywhere. In the more intact part of the muscle, between the fibers, the macrophages are often spindle-shaped or stellate. Many of these are doubtless the so-called resting-wandering cells or clasmatoocytes. In all areas, but particularly where the tissue is fragmented, cells of more rounded or oval shape are found. These are similar to those described for the second day, but are much more abundant.

It is at once seen that there are great variations in the dimensions of the macrophages. The largest are enormous cells, studded with granules of irregular size; the average long diameter of 10 of these was 23.3 microns. They are distinctly more voluminous than the greatest macrophages of the second day and contain more dyestuff, thus indicating a gain in phagocytic efficiency. Their shape varies; some are rounded or oval, while others are very irregular in outline—elongated, with long processes sometimes constricted from the main part of the cell. They resemble the “polyblasts” which Tschaschin found in areas of inflammation in the rat vitally stained with isamin-blue and in the rabbit vitally stained with trypan-blue. The cytoplasm, aside from the dye-granules, is for the most part clear.

It is significant that there are all degrees of size in the dye-containing cells, from this extremely large macrophage to quite small cells which harbor very little dye; this type again grades off into a small round cell with a relatively large nucleus and but little cytoplasm, which resembles a lymphocyte. In the carmine-stained preparations they present a large, deeply staining nucleus and little cytoplasm. They contain no dyestuff. We may select transitional cells from almost any part of the field and arrange them in an order of size, as in figure 8. From the appearance presented by such a series it will be at once inferred that an hypertrophy of the cytoplasm of this small round cell has taken place and that coincident with this hypertrophy there has been an acquisition of the power of phagocytosis; for dye-granules, at first small in number and size, begin to make their appearance in the enlarged cytoplasm. These granules gradually become larger and more numerous as the cytoplasm increases in size, and reach their maximum development in the largest polyblasts. There seems to be a direct correspondence between the size of the macrophage and the amount of dyestuff it contains, on the one hand, and the extent of its phagocytic activities on the other; for it may be assumed that the reaction of the phagocyte toward the dyestuff—in degree as well as in kind—is an index of its behavior toward the material which it is specialized to ingest.

During this metamorphosis the nucleus undergoes little change in size, so that it is extremely small in comparison with the enormous mass of the cytoplasm. In the early changes of the lymphocytoid cell the nucleus often becomes bilobed or even binucleate. Superficially it may resemble a polymorphonuclear leucocyte. In the mature forms no multinucleate or giant-cells were observed.

The transitional stages which have been described suggest that at least some of the macrophages have been derived from the lymphocyte-like cells and this

hypothetical origin will be discussed later. Mitotic figures were extremely rare among these cells; thus it appears that the cells do not multiply in the fracture-area, but increase by immigration. It is of interest to observe in this connection that in the capillaries of the region of macrophage invasion there are large numbers of lymphocytes; these seem to be present mainly in the large sinusoids. Bay-like diverticula from the capillaries, representing the young vascular sprouts, were observed crowded with lymphocytes, and it may be that these represent important points for the exit of the embryo macrophages from the blood-stream, and the circulation stasis here would be favorable to such a proceeding. The finding of a small clump of lymphocytes in the tissues at the apex of one of these vascular pouches lends support to this supposition.

That there is an actual destruction of effete tissue is shown by the progressive disappearance of this in the consecutive stages; and this tissue-erosion is coincident with the presence of increased numbers of very large macrophages. Indeed, as will be seen, the numbers and size of the macrophages in a given area are a good index of the degree of protoplasm demolition occurring therein; in other words, there is a direct parallel between the exaltation of the potentialities of the macrophagic tissue and the absorption of the products of proteolysis.

Though the macrophages are found at times lying quite close to—even in contact with—the tissue undergoing lysis, their characteristic position is rather one of complete separation from such tissue, for most of the cells are to be seen lying free in the colloidal fluid which bathes them. This fluid, which stains faintly pink with eosin, is necessarily heavily charged with the products of proteolysis. The macrophages are thus favorably situated for functioning in the absorption of this waste material.

In the third-day stage a macrophage is occasionally found in which, though the cytoplasm is voluminous, the dye-granules are relatively few and scattered. The cytoplasm presents a vacuolate appearance, and in some cases one gains the impression that the cell is filled with phagocytized tissue-waste. It is not unlikely that these cells have been active for a longer period than the well-stained macrophages. They are most numerous in the areas of young scar tissue—where tissue destruction is largely over. Small and intermediate macrophages, as well as the larger sizes, show this meager type of staining. A few seem to be falling to pieces.

Compared with the typical macrophages these exhausted or involution forms are as yet insignificant in number. They are more abundant in the later stages, as will be seen.

Polymorphonuclear leucocytes were present in appreciable numbers in the tissues of the fracture-region. They were somewhat irregularly distributed and were often quite closely associated with heavily stained macrophages. Though favorably situated for imbibition of the dyestuff, being at rest or wandering in tissue-spaces whose fluids were thoroughly impregnated with it (Downey, 1917), yet no dye-granules were found in them. Thus, if this cell functions in the absorption of colloids it must do so very sparingly. It also differs from the macrophages in

that it does not undergo cytoplasmic hypertrophy; indeed, the only noticeable change in it is the usual increase in lobulation of the nucleus, this structure being quite complex at times.

The presence of these neutrophils in an area of inflammation, even of the aseptic type, which we have here to deal with, is of course to be looked for, and it may be that one of their functions here is the elaboration of proteolytic enzymes; more commonly they are found closely associated with the disappearing tissue than in the open spaces.

Fibroblasts were fairly abundant throughout the wound-area. In some of these a few fine round dye-granules were observed; the most, however, appeared unstained.

The *skull sections* show substantially the same picture as the long bones. There is a good deal of fragmented and pulped débris which stains diffusely and densely blue, thus confirming the findings for this specimen in the gross *cleared* condition. This material is the bone dust and other tissue injured in the operation. There is also quite a lot of extravasated blood and exudate. Though macrophages were not discerned in the cleared specimen, in the sections they are very numerous, but the picture is hardly as striking as in the long bones. Transitional types of dye-containing cells are seen, as in the long bones, but are less abundant. Distinct staining of the ends of the cut bone is present. Polymorphonuclear leucocytes are fairly numerous, but contain no dye, even where they appear among the densely staining débris.

On the third day the sections, like the cleared ribs, show the *callus* somewhat thicker and more vascular than on the second day. It is particularly well seen in the sections of the long bones. There is much more ossification evident than heretofore, and distinct trabeculae, staining like bone and containing definite bone-cells, project into the outer, more cellular layer. Somewhat larger spaces, with osteoblasts lining their walls here and there, occur between the trabeculae. Many of these contain capillaries of small caliber. The largest spaces are situated close to the original bone. Often it is evident that they communicate with the Haversian canals of the bone, which have apparently undergone an enlargement in the regions close to the callus. Frequently such a space is found to contain a blood-sinus, and in some spaces there may be situated a multinucleated basophilic cell resembling a mass of osteoblasts. These cells are probably the so-called "osteoclasts." They are not numerous, and no dye-granules were found in them.

When the cleared and uncounterstained section is looked at with the low-power the callus has a pale-bluish appearance with a few spots of darker blue representing scattered cells. Under the oil immersion these cells are found to occupy the interstices between the young bony plates; they are reticular cells, and in them it is possible, by close scrutiny, to see a very faint blue granulation. They are to be regarded as the young recruits of the army of macrophages which will inhabit the expanding callus spaces in the later stages. They are largest and their granulation is most marked in areas near the original bone—indeed here, especially

in the dilated extensions of the Haversian canals, a cell well studded with dye-granules is often encountered. In size and general appearance these are quite similar to the macrophages which inhabit the actively growing regions of developing bone described by Shipley and Maeklin (1916<sup>2</sup>), as shown by a comparison with the specimens of these authors, and also with sections from the end of a growing bone from the same animal (S 11-1) from which the fracture-sections were cut. In the Haversian canals of the adjacent normal bone, cells containing dye-granules are rare; when they do occur they are of small size and slight degree of staining. In no case are the trypanophil cells of the callus at this stage at all comparable in size and staining intensity with the extraosseous phagocytes of the degenerating tissues; indeed, when compared with these they are quite insignificant.

As to the function of these trypanophil cells, it is noteworthy that some slight breaking down of tissue occurs in association with the hollowing-out of the intertrabecular spaces, and it may well be that they are being developed to absorb the waste products resulting from this process. In any event their advent in the spaces is coincident with the enlargement of the latter. Of even greater significance, however, is their importance in these early stages as examples of phagocytic preparedness, for, as will be later seen, it is these cells which light up suddenly into vigorous action coincidently with the onset of very active bone resorption, which begins about the tenth day.

The source of the trypanophilic cells in the spaces of the callus would seem to be the tissue of the Haversian canals of the old bone. They are doubtless of the same type as the reticulo-endothelial macrophages found in ordinary bone marrow. Under the conditions of bone erosion they have increased in size and phagocytic ability, for their well-developed representatives are obviously much larger and more deeply stained than the cells of the non-growing bone marrow.

In the third-day stage, then, it is evident, judging from the structure of the bone, that osseous resorption is under way to a limited extent in the Haversian spaces immediately underlying the new callus and in the spaces of the callus itself. It is significant that trypanophilic cells should make their appearance in the spaces which are being hollowed out simultaneously with the onset of this process and that there should be a direct relationship between their size and staining intensity, on the one hand, and the extent of the bone-excavation on the other.

The callus of the skull, though not so far advanced as that of the long bones, is of the same general character.

Summarizing the features of the staining at the third day, we may first mention the immense congregation of macrophages which are evidently engaged in clearing away the debris consisting of blood-clot, damaged muscle, bone, and other tissues. The great majority of these are apparently developed from the lymphocyte-like cells, brought by the blood-stream to the site of the fracture, where they rapidly gain in size and phagocytic power. Their function is the phagocytosis of waste products from tissue breakdown. Diffuse staining of the defunct tissue is present. In the expanding spaces of the growing callus trypanophilic reticulum cells have

appeared, which are as yet, for the most part, not strongly stained; to these an important phagocytic rôle is assigned.

FIFTH-DAY STAGE. (S 12-1).

On the fifth day the *cleared* preparation of fractured *ribs* shows the macrophagic sheath still very conspicuous about the area of the wound, its blue lines of trypanophil cells stretching out into the muscle and invading the spaces between the fibers. Within it the clear zone of young callus is somewhat increased. The staining of the ends of the bones is less marked, and it is probable that this is because the dead material is being cleared away; there is also less blue *débris* around the bone. Large macrophages are seen about the ends of the bone and in the entrances of the marrow cavity. The macrophages are at least as numerous as on the third day and are distinctly larger in size, being quite conspicuous even under the low power of the binocular.

In the *sections* of the *long bones* hosts of macrophages throng the areas of effete muscle and other tissue damaged by the trauma. Diffusely staining damaged tissue is found, but is somewhat less marked. There is a slight amount of extravasated blood and fluid exudate. The macrophages are very large and are loaded with dye-granules. There are not so many transitional types as in the third-day stage. They resemble in form those already described. The exhausted or involution forms, seen now and then in the third-day stage, are here somewhat more often found; as before, they are much more frequently encountered in scarring areas. In some the cytoplasm presents nothing more than a mere network in which an occasional dye-granule is seen.

Some of the cells show a very few large granular masses, suggesting that the smaller dye-granules have coalesced; occasionally a macrophage is encountered in which the nucleus stains diffusely, thus pointing to the death of the cell (MacCurdy and Evans, 1912). These results may be due to an overloading of the cell with dyestuff. Sometimes, too, diffuse staining of cell inclusions leads to such optical effects. Similar cell-staining has been described by other authors, as Tschaschin (1913).

Polymorphonuclear leucocytes are not so numerous as on the third day. They were not found to contain dye-granules, although this animal was subjected to the vital dye for 72 hours.

Scar formation is in progress, and the fibroblasts are of a more mature type. Rarely indeed are dye-granules, even of small size, to be found in them.

In the *skull sections* of the fifth-day stage there is still some damaged tissue, taking a diffuse blue stain. The ends of the bone are tinged, but rather less strongly than before. Dead fibers of muscle and connective tissue stain bluish and show stained nuclei. There is some extravasated blood and fluid exudate.

In association with this *débris*, macrophages are abundant, but are not so marked as in the long bones. As in the long bones of this stage, there are comparatively few transitional types.

The *callus* of the fifth day shows evidences of development in its greater thickness and in its longer trabeculae of bone. The spaces between the bony plates are larger, and a pronounced feature of this stage is the presence of large blood-sinuses within these spaces, especially in the region next to the original bone. They are distinctly more capacious than those of earlier stages. Many of these sinuses have strands of endothelium projecting into the lumen, or even completely crossing it; again, the wall is often irregular and instances are easily found where a smaller capillary is being incorporated into a larger one. Some of the intertrabecular spaces show several small capillaries, and it is evident that the larger sinuses are formed from the fusion of two or more smaller vessels.

As in the third-day stage, cells faintly stained with small blue granules are found in the spaces of the callus. They are somewhat more numerous and a little more distinctly stained than in the earlier stages, and are largest and most strongly stained in the intervals between the walls of the blood-sinuses and the bone, and hence the area close to the original bone is most generously supplied with them. Often the endothelial cells contain dye-granules. None of these cells are at all to be compared in size and strength of staining with the large phagocytes of the degenerating tissue.

The same parallel is thus present here as in the last stage, viz, the excavation of the spaces of the callus which goes hand in hand with the number and phagocytic ability of the trypanophilic cells.

Quite a lot of cartilage is found in the callus of this stage, but it shows nothing of interest from the standpoint of vital staining.

Summing up the fifth-day stage, the most striking feature, as at the third day, is the great number of large macrophages vigorously at work in clearing out the waste material at the site of the injury. There is not such an active development of macrophages, for fewer transitional forms are seen. That defunct tissue is being cleared away is evident from the decreased amount of diffusely stained debris. Fibrous tissue is developing.

Trypanophilic reticulum cells, showing a little increase in numbers and staining powers, are found in the expanding callus spaces and often envelop the large thin-walled blood-sinuses, which are now a prominent feature of the larger spaces.

#### SIXTH-DAY STAGE. (S 11-2).

The sixth-day stage as seen in the *cleared ribs* is characterized by the same dense cloud of large macrophages enveloping the site of the fracture. Plugs of macrophagic tissue fill the open ends of the bone. The broken surfaces appear to stain only faintly and also to be becoming round and thin; in this we have evidence that the dead bone is disappearing. Debris is slight in amount. The callus is more sharply outlined and is optically denser.

The *cleared skull* shows the same local increase in the trypanophil cells, the space between the insert and the regions about the edge of the trephine opening being literally crowded with them. Some diffusely staining debris is seen.



The *sections* of the *long bone* show dense collections of macrophages of similar type to those of earlier stages (fig. 9), except that there are not nearly so many young transitional forms, and there are more degenerate cells in places. Wherever there is fragmented tissue to be absorbed, especially shreds of muscle, there are myriads of large macrophages stuffed with blue granules (fig. 10). The crevices between muscle fibers are often crammed with veritable nests of these phagocytes. As formerly, the damaged tissue stains diffusely blue. There is very little exudate.

It is evident, however, that in some regions tissue destruction is waning. Large patches of young scar tissue appear (fig. 9, s), and in the sections simply cleared without counterstaining one has a striking demonstration that in such areas the phagocytes are much less prominent—indeed, they are often relatively inconspicuous. In figure 9 an area of scar tissue appears contrasted with an area of degenerating muscle fiber, and it is easily seen that in the former the blue staining is very weak, whereas it is exceedingly marked in the latter. The macrophages may be looked upon as assisting to prepare the way for the scar tissue, and they are thus an important factor in repair.

Upon searching with the high-power lens through the patches of young fibroblasts (fig. 9, s), one is struck with the great number of phantom-like cells, which appear to be macrophages undergoing degeneration (fig. 11). They resemble similar cells described for the third and fifth days, but are much more numerous. Their appearance is very characteristic; they often resemble a mass of fishing-net, the knots being represented by the scattered dye-granules. The cytoplasm, at first pale and vacuolated, is in the more extreme types reduced to a mere lacework, and here and there in this are to be found occasional irregular grains of blue, showing that the specific function has not altogether departed. Not infrequently cellular inclusions are found in them, often stained diffusely blue. The cytoplasm may be reduced to tattered remnants. Fragments of cytoplasm, containing a few scattered dye-granules, are sometimes found, marking the remains of a cell which has suffered dissolution. Often the nucleus is stained blue—an evidence of cell death.

The forms of degeneration which have been described are the most extreme. The vast majority of the macrophages in the scarred areas on the sixth day are in this condition. There are, however, cells which are not so advanced in degeneration, especially in regions where fibroblasts are less abundant. These contain more dyestuff and usually are less vacuolate. All grades of these are found connecting the normal macrophage at the one extreme with the disintegrating remnant at the other.

The reason why these cells drop out of the ranks and break up may be because they have become exhausted in the course of their strenuous activities, for often their cytoplasm is clogged with phagocytized material; they are thus incapacitated for ingestion of the dyestuff. Possibly, too, the cells have become intoxicated by the materials taken up. Again, since these cells are found characteristically in the scarring areas, where waste material has been removed, and consequently where the opportunity for activity—even the stimulus thereto—has departed, it may be



that they have atrophied from disuse or perhaps from the encroachment of the vigorous young fibroblasts. It must be borne in mind that these degenerate forms are occasionally found in areas of active proteolysis, associated with densely stained cells, and that they give evidence of some phagocytic activity. They do not appear all to be old cells, for some of them are quite small; young cells, however, as well as mature ones, could, theoretically, fall victims to overwork, poisoning, disuse atrophy, or fibroblastic overgrowth.

The subsequent history, then, of an area such as that shown in figure 7 of the third day is as follows: while the fibroblasts go on developing scar tissue the macrophages complete their work of clearing away the products of protoplasmic solution. This being done, they gradually disintegrate *in situ*. Certain it is that these cells lose their power of motility and literally die in their tracks. Becoming reduced to mere cell skeletons, they collapse, break up, perhaps under the influence of the enzymes of the neutrophilic leucocytes, and doubtless pass off in the tissue fluids. It is quite possible that any solid fragments that remain fall a prey to the neighboring phagocytes. This fate seems to overtake most of the macrophages.

Polymorphonuclear leucocytes were not infrequently found in this specimen, in areas where damaged tissue and macrophages were present. They were not vitally stained.

The *skull sections* of S11-2 present no additional points of importance. The picture, though similar, is much less striking than in the long bones, the macrophagic tissue being comparatively slight in amount, although increased over normal.

The *callus* of the long bone of S11-2 is still more extensive on the sixth day, and consists, as before, of a rather delicate network of trabeculae inclosing spaces. The spaces of the interior, especially near the old bone, show enlargement, so that there has been some tissue destruction here. The trabeculae are not much thickened.

Under the low-power there may be seen, in the cleared uncounterstained callus, many more trypanophil cells than in earlier stages, and they are a little more distinctly stained, especially in the interior of the callus. As before, the dyestuff is less obvious in the younger outlying tissue. The trabeculae are more dense and show a fibrous structure.

Under the high-power the peripheral spaces, smaller and of more recent origin, are filled with cells and contain small capillaries. Farther back the somewhat older spaces are larger and in them is noted a loose, plexiform aggregation of cells (fig. 12). As development proceeds, it is evident that the cells of the spaces separate to form a large-meshed reticulum, while the capillaries become larger. Later still, the capillaries coalesce to form sinuses (fig. 12 BS). While this is going on, either growth or breakdown of the trabeculae may take place. If the former, the walls appear lined with osteoblasts; if the latter, these cells are absent and the walls of the trabeculae are roughened. Both processes may be going on in the walls of the same space. As has been noted, the reticulum cells are often phagocytic, as shown by their trypanophilic reaction (fig. 12 M), and it is apparently in association with the process of disintegration of areas of callus that these cells develop their gor-

mandizing properties. Although reticulum cells containing a few small blue granules are seen in the younger spaces, the staining is stronger and the cells more evident in the older spaces. Here the dye is fairly well marked. There has been no increase in size, and but little in staining intensity as compared with the third and fifth day stages. The blue-stained cells frequently lie between the endothelium of the blood-sinuses (fig. 12 BS) and the trabeculae of young bone (fig. 12 c). The endothelium itself often contains granules of dyestuff. The vital-staining is nowhere at all comparable with the brilliant coloration of the extraosseous macrophages. The reticulum cells are very small as compared with the larger macrophages, and the dye-granules are also comparatively small.

A feature of this stage, as shown in the carmine-stained preparations under the oil-immersion lens, is the large number of mitotic figures which occur in the trypanophilic cells (fig. 13). These are especially numerous in the outer regions of the callus, where the cells are multiplying rapidly. They are reticulum cells, and it is evident that their manner of multiplication is by karyokinesis. The presence of dye-granules within the cytoplasm is not incompatible with mitosis, as was shown for the Kupffer cells by Evans, Bowman, and Winternitz (1914).

Giant-cells are extremely rare. Only one small giant-cell was found after an extended search. No dye-granules were to be seen in it.

In the *skull sections* of S 11-2 callus is slight in amount. Reticular phagocytes are beginning to appear in it.

The *other member* of the six-day stage, S 13-1, shows essentially the same features. The callus occupying the marrow cavity is particularly well developed and innumerable trypanophilic reticulum cells are found in the intertrabecular spaces. The original bone in the vicinity of the callus has a worm-eaten appearance; in these spaces the vitally stained reticulum cells are quite conspicuous and numerous. In addition there are here a fair number of giant-cells, some of large size. None contain dye-granules. The blood-sinuses of this region are very large.

In brief, then, it is noted that at the six-day stage there is the same relationship apparent between resorption of the callus and the presence of trypanophilic cells. Of special interest is the presence of well-stained cells in the spaces which have been eroded in the old bone. The obvious relationship between the large blood-sinuses and bone-resorption is also noteworthy.

In reviewing the sixth-day stage we have to note the persistence (in areas where tissue destruction is evidently proceeding rapidly, especially in moribund muscle) of enormous numbers of large and very phagocytic macrophages. They are of the same type as in preceding stages, except that transitional forms are much less frequently seen than on the third day, so that fewer young cells are being called out. In areas where destruction of tissue has ceased scar-formation is well under way, and here the loose fibrous tissue contains immense numbers of relatively weak-staining but often voluminous macrophages, which appear to be degenerate or involution forms. A few of them are found among the active phagocytes. Thus, in this stage, tissue resorption is gradually ceasing, as shown by

diminishing evidence of débris and by the extensive scar-formation, but in some areas it is still proceeding actively, as attested by the remaining tissue-waste, engaged by the persisting hordes of active phagocytes. The enlarging callus shows an increase in number of the trypanophilic reticulum cells and some heightening in their staining activity, especially in the older spaces.

NINTH-DAY STAGE. (S 17-2).

In the *cleared ribs* of the ninth day (fig. 4) no diffuse staining of the ends of bone, or the surrounding injured tissues, can be made out. Hence it may be inferred that the clearing away of débris and exudate is at an end. As would be expected, this process is completed sooner in a small bone like the rib than in a larger bone, such as the femur, where the tissue damage is, of course, much more severe.

The investment of trypanophil cells about the fracture-area in the cleared ribs is still quite obvious (fig. 4), though less conspicuous than in the earlier stages. As will be seen from later specimens, these cells persist at the site of their former labors in the fracture-area for a few days after tinctorial and histological evidence of damaged tissue and exudate has disappeared. They gradually dwindle in numbers and vital-staining ability.

The edges of the new callus are much more sharply defined and are distinctly seen throughout. In addition, the callus is considerably denser and is reticular in structure, an appearance which may be interpreted as evidence of extensive ossification. In the rib from which the figure was made this reticular structure is complete throughout, but in the others it is incomplete between the bone ends. Apparently, movement has delayed ossification in these cases.

In the *cleared skull* of the ninth day the bony insert of the left side slightly overlaps one of the edges (fig. 6). This insert consisted of sterile dead bone from another rat. On the right side the space is unfilled. This opening crosses the mid-line and occupies some of the territory of the left parietal bone. A little bluish débris is seen lying about. The edges of the bone are slightly blue. Clouds of macrophages infiltrate the membrane filling the open spaces, as shown in the drawing. The clear spaces in this represent areas of extensive scarring; here the macrophages are much less conspicuous. Some unstained ossified callus radiates from the edges of the openings.

The *sections* of this stage are not good.

The principal features to be noted on the ninth day are the gradual subsidence of the extraosseous macrophages and the occupation of their fields by the scar tissue. In the callus increasing density is evident.

TENTH-DAY STAGE. (S 5-1), (S 6-1), (S 6-2).

In the *cleared skull* of the tenth day (S 5-1) a local excess of macrophages is still evident, as well as a little stained débris. In the *sections* of this specimen the diffuse staining is found to be due to the presence of a small abscess, the result of an infection. The exudate has taken a diffuse blue color. Macrophages are found in numbers in the periphery of this abscess. Some appear degenerate. Dyestuff was not found in the polymorphonuclear leucocytes of the abscess.

The *sections* of the *long bones* of the tenth day (S6-1, S6-2) present very little remaining débris. Occasionally a few shreds of muscle are found, showing evidences of degeneration. Here macrophages are fairly numerous, though much reduced in number as compared with earlier stages. These phagocytes are of the mature type, with very few transitional stages. Comparatively few involution forms are seen. Scar-formation is advanced.

The *callus* of the *long bones* (S6-1, S6-2) at this stage shows little increase in volume. The most interesting feature is seen in the hollowed-out spaces near the original bone; for here, especially in the cleared and uncounterstained sections, one is struck with the conspicuous appearance of the many vitally stained cells. Even under the low-power they are very evident, as shown by figure 14, drawn from a section from S6-1, and are much more brightly stained than in the earlier stages. As before, they are often distributed around the thin-walled blood-sinuses. They are not in actual contact with the callus. They are found in the callus of the marrow cavity as well as in that of the external surface of the bone. The degree of staining intensity gradually diminishes as the outer district of the callus is approached, and in the more compact areas of callus, where apparently no resorption is occurring, the blue cells are very few and insignificant in staining.

A description of the general morphology of the typical reticulum macrophages, as seen with the high-power lens, may here be given. It applies, with slight variations, to the cells of the succeeding stages up to and including the twentieth day.

Like all reticulum cells they are characterized by a number of processes—flat and wide, or extended and threadlike—through which they are directly continuous with their neighbors. The cell-body is oval or stellate, and often elongated and flattened. The nucleus is fairly large and usually rounded or of oval outline, though it may be notched.

The trypanophilic reticulum cells at the tenth-day stage are the same as at the earlier stages, except that they are often much more brilliantly stained and hence may be regarded as much more actively phagocytic. They are quite evidently true reticulum cells, and not invading elements from other regions of the body, for their protoplasmic connections with other reticulum cells, which may or may not contain dye-granules, can easily be made out. The size of the well-stained cells varies; the tendency seems to be to develop a cell of fairly uniform dimensions. The average diameter of ten of the largest cells at the tenth-day stage, as taken between the widest extremities of the dye-granule content, was found to be 7.6 microns. They are thus considerably smaller than the largest extraosseous macrophages, though larger than the macrophages of ordinary bone marrow. The arrangement of the cells in the spaces is the same as that in stages already described, or as in the later stages.

The degree of staining varies considerably; some cells have a mere sprinkling of small dye-granules, while others are stuffed full of granules of larger size (fig. 15, *a*), and there are all degrees of variation between these extremes. Usually the nucleus is completely inclosed in a zone of blue granules, but not infrequently cells are

found in which areas of cytoplasm are without visible dyestuff. In the typical phagocytes of this stage the granules are much larger than in the earlier stages, and there are more of them. There is much more dyestuff in the cell.

Some of the cells contain a very few quite large granules of dyestuff instead of a multitude of smaller ones, suggesting that there has been a coalescence of the latter. Again, in an occasional cell, the appearance is as though the entire nucleus were stained, pointing to the death of the cell. It is possible that these cells have succumbed to the action of the material ingested; or such appearances may be due to stained protoplasmic inclusions. Similar findings have been noted in extraosseous macrophages.

As the cell fills with its pabulum or with dye it becomes more rounded and the processes are reduced to fine threads. As a rule, the dyestuff does not find its way far into the processes, so that the dye-granule contour is usually oval, as is seen in figure 15, drawn from representative uncounterstained reticulum macrophages of different periods.

A few polymorphonuclear leucocytes are seen associated with the macrophages. No dyestuff was found in them. Osteoblasts are sometimes met with in their vicinity, but never take the dyestuff. Giant-cells were not encountered.

In the *skull* of this stage (S 5-1) distinctly stained reticulum cells were found in the spaces in the callus and also in the old bone.

As will be seen from the examination of the subsequent stages, the resorption of the callus is most active during the period from the tenth to the twentieth day or shortly after; before that period the main trend in the callus is constructive rather than destructive, although, as has been pointed out, there is, even during this evolutionary phase, some hollowing-out of the spaces, as seen, for instance, in the specimens of the fifth and sixth days. Furthermore, the stages from the tenth to the twentieth day show that the greatest amount of resorption occurs at first in the vicinity of the old bone, for the spaces there undergo the greatest expansion. It is thus evident that the most brilliantly stained, and hence the most phagocytic, cells are found where callus destruction is most active. It is plain, too, that these cells have undergone an exaltation of their powers of phagocytosis coincidently with the acceleration of callus resorption.

Reviewing the tenth day it is noted that extraosseous macrophages are gradually disappearing, as shown by the many degenerate forms and by the relative inconspicuousness of the survivors. Their place is taken by scar tissue. In the callus the most striking point is the marked hypertrophy and increase in phagocytic ability of the macrophages of the reticulum of the callus spaces undergoing expansion at the expense of their osseous walls.

#### TWELFTH-DAY STAGE. (S 5-2).

Little or no stained débris was noted in the *cleared skull* of the twelfth day, nor was it found after this stage. In this specimen the opening in the bone was left unfilled. The central part of this opening is almost clear, being occupied by

scar tissue, in which but few macrophages may be described. Surrounding this scar, and bordering the bone edges, there remain macrophages in considerable numbers. *Callus* in this specimen is well advanced, and in the spaces of this, which are now quite large, considerable numbers of large-sized macrophages may easily be seen with the higher power of the binocular.

The *sections* of the *skull* show marked scarring, but nothing of note as to vital staining. In the *long-bone sections* no blue-staining debris was noted. In the vicinity of the fracture the muscle fibers are scattered, and between them there is a great deal of scar tissue. Here, too, there is some excess in the number of macrophages, but these cells are numerically insignificant as compared with earlier stages. Transitionals and degenerate forms are very rare. It is evident that little or no tissue destruction is going on. The synchronism between proteolysis on the one hand and heightened efficiency of the macrophagic tissue on the other is thus consistently maintained to the end, both phenomena ceasing practically at the same time. As will be seen by the subsequent stages, there is no further evidence, beyond the twelfth day, that extraosseous tissue destruction is going on, and the macrophages which remain in places where tissue resorption has once taken place soon disappear.

The *callus* is of greater dimensions than before, and is evidently still growing in places, as the outer layer of deeply basophilic osteoblasts is often seen to be quite thick. It is very obvious that erosion of the callus is proceeding rapidly at this stage, for not only do the spaces in the vicinity of the original bone show increase in size, but the spaces throughout the callus almost as far as the periphery are enlarged. These spaces are occupied by large, very brilliantly stained reticulum macrophages. The cells are very numerous and crowded, some fields being quite blue with them, so that they present an exceedingly striking appearance in the cleared section (fig. 15, *b*). They are even more marked than in the last stage, and have evidently developed an intense avidity for the blue dye, for the cytoplasm is literally packed with granules in most of the cells. There are degrees of staining, however, and some cells are found with but a sprinkling of dye-granules, so that it may be assumed that as time goes on more and more reticulum cells are developed as phagocytes. Again, some of the cells have a few very large, rounded granules, looking as though they were produced by a concentration of dye from the rest of the cytoplasm, as was noted in the last stage.

The distribution of the macrophages is more widespread than before and goes hand in hand with erosion of the callus, for they inhabit the expanding spaces almost as far out as the periphery. In size they show a slight increase over those of the tenth day, the average long diameter of ten of the largest cells being 9.15 microns. These are fairly uniform in dimensions. As before, they are found in the loose reticulum of the spaces (fig. 16), or are crowded between the plates of bone and the walls of the sinuses. They are not in actual contact with the bone. The blood sinuses are very large at this stage, pointing to a sluggish blood flow. Their walls are formed by a single layer of endothelium.

Since the macrophages are largest, most phagocytic, and most numerous where resorption of the provisional callus is going on most rapidly, it is quite evident that they are concerned very intimately with the clearing away of the callus.

A thickening of the trabeculae which escape erosion is evident, and thus there have been going on, side by side, the antagonistic processes of bone-erosion and bone-building.

Giant-cells are sometimes found, but contain no dyestuff. Large areas of cartilage are seen in this specimen. Marrow tissue is present in some of the spaces.

In the callus spaces of the skull macrophages are found, as in the callus of the long bones.

Passing in review the twelfth-day stage, there is to be noted an almost complete cessation of tissue destruction, and the extraosseous macrophages are but little increased over the normal. The callus, on the other hand, shows an exaltation in staining and increase in size on the part of the macrophagic inhabitants of the areas where bone destruction is actively proceeding. Bone-erosion and bone-building are combining to give shape and strength to the permanent callus.

#### THIRTEENTH-DAY STAGE. (S 11-3).

The thirteenth-day stage shows essentially the same conditions. The staining here is not good. In the *cleared ribs* no evidence of damaged tissue is present. Macrophages are much reduced in number and staining intensity. Callus is well advanced.

In the *cleared skull*, owing to the poorness of the staining, neither stained debris nor extraosseous macrophages can be made out. The *callus* is well marked and a few blue-stained cells are seen in its spaces.

#### FIFTEENTH-DAY STAGE. (S 5-3).

In the *sections* of the *long bone* of the fifteenth-day stage there is no diffusely staining material to be seen. In the vicinity of the fracture, where tissue destruction has been proceeding, the muscle-tissue is loose, and the scattered fibers are interspersed with scar tissue and macrophages. Transitional types are very rare. No degenerate forms were noted, nor were any found in later stages. Scar tissue is abundant. It seems to be evident that resorption of dead tissue has ceased. The extraosseous staining presents a much less striking picture than in the earlier stages, and from this period it becomes less and less noteworthy.

The *sections* of the *skull* show no remaining dead tissue. It is not evident that there is any excess of macrophages at the site of the healed wound.

The *callus* of the *long bones* of S 5-3 is quite extensive, but, judging from the almost entire absence of typical osteoblastic formations on the periphery, its expansion has practically ceased. The regions near the original bone are characterized by thickened osseous trabeculae, often inclosing large tracts of loose cellular marrow tissue, traversed by voluminous thin-walled blood-sinuses. Some macrophages are found here, but in regions where the marrow tissue has become well established

they are relatively few and inconspicuous. The apparent reduction in their number here is due in part at least to the increased proportion of other cells as well as to the fact that the tissue becomes more open in structure. The phagocytes are to be regarded as having a hand in the preparation of the marrow-tissue habitat.

Farther out in the callus the spaces are large, and many are occupied by enormous blood-sinuses with walls composed only of endothelium. Here many of the trabeculae are thin and are evidently being broken down; others are thickened and are being built up. This is the most active area of bone resorption. In this region hordes of reticulum macrophages, brightly stained and of increased size, form a striking picture and are the most outstanding feature of this stage. Their district of greatest concentration, as compared with the last stage, has shifted peripherally, in company with the district of greatest callus-erosion. The preference of the macrophages for regions of bone resorption is all the more significant because it is consistent with the behavior of these cells in earlier stages.

The morphology of the macrophages and their relationship to the surrounding structures need no special description here, for they are similar to those of the last two stages described. Careful measurements of the longest diameters of ten of the largest cells gives an average length of 9.95 microns—thus showing that the cells have become somewhat hypertrophied. The dye-granule content is similar to that of earlier stages (fig. 15 c). Some associated polymorphonuclear leucocytes are found in the tissue-spaces, but no dye-granules appear in them. Giant-cells are quite frequently found, but they also contain not a trace of dye. Often they give the impression of being a mere scrap-heap of old osteoblasts and bone-cells, the residue of bone-erosion (Arey, 1917).

In the *skull* of this stage macrophages of large size and conspicuous staining inhabit the *callus* spaces. Large blood-sinuses in the spaces are also a feature here.

On the whole it may be concluded that at the fifteenth day little or no tissue is being destroyed outside of the bone, for the number and staining intensity of the macrophages have been reduced almost to normal and there is an absence of demonstrable moribund tissue. In the callus, however, the reticulum macrophages are especially abundant and phagocytic in the regions of bone destruction, and appear to be actively engaged in phagocytizing the products of this process.

#### TWENTIETH-DAY STAGE. (S 12-2), (S 13-2).

The *cleared ribs* of the twentieth day (fig. 5) are very different from those of earlier stages, as the third and fifth days, for the blue investment has become very thin and but few granules, representing macrophages, are to be seen in it with the binocular. What blue there is seems to be largely a diffuse staining of the periosteum and scar-tissue. The size of the fracture-site is much reduced and the callus has undergone a good deal of resorption, the part now being of a slender spindle-like form. The interior of the bone is occupied by a large-meshed callus, containing some blue cells, and the medullary canal is evidently being restored. Other ribs at this stage show less perfect approximation, but essentially the same features.



In the *cleared skull* of the twentieth day the extraosseous macrophages show little if any increase above normal. The membrane of connective-tissue filling in the intervals between the bone pieces stains diffusely blue, especially where it is thickened near the edges, but this staining is not strongly marked and resembles the staining of any similar fibrous connective-tissue, as tendon (foot-note, page 8, c). A few persisting macrophages appear in it. The new bony callus is abundant and in its spaces a few trypanophilic cells are seen.

In the *sections* of the *long bones* of the twentieth day (S 12-2, S 13-2) large areas of callus and of scar-tissue are conspicuous. Near the fracture the muscle-fibers, as noted for the two last-mentioned stages, are often scattered and surrounded by the new scar. They are frequently rounded and dwarfed. Numbers of macrophages still persist in places such as this; probably they are not actively functioning and gradually disappear, for, barring a few found at the thirtieth day, they are not encountered in future stages. No evidence of extraosseous proteolysis is to be seen, there being no blue-staining debris or degenerate tissue. Transitional cells were not found in the twentieth-day stage.

The *callus* of the long bones (S 12-2) of the twentieth day is especially interesting. At no stage are there greater evidences of erosion. Enormous spaces characterize the cross-sections and in some of these but a mere shell of bone remains at the periphery, while the central region is filled with marrow-tissue, tunneled by large blood-sinuses. Again, in other sections the spaces are partitioned by stout trabeculae of bone. A corner of a typical section is presented in figure 17. Here the most spectacular feature, brought out with singular sharpness in the cleared section, is the multitude of brilliantly stained reticulum macrophages at work in the smaller spaces. As a rule they are now massed in the more peripheral recesses, though some crowd into corners here and there throughout the bony spongework, where temporary osseous scaffolding still awaits removal. A few haunt the interstices of the marrow tissue. In many areas they are very inconspicuous or absent. Here bone erosion has evidently ceased. It is obvious that the area of most active bone destruction has shifted from the central mass of the callus to the more outlying regions and, as in the last stage, the macrophagic army has kept pace with this onward march.

Striking indeed are the pictures presented under the higher powers. In figure 18 is seen a small field, magnified 190 times, from the section from which figure 17 was taken. The formations of macrophages (M), as before, are drawn up in the perivascular spaces or deployed through the loose reticulum. In these areas of most active bone-erosion the phagocytes are even larger in size than at the fifteenth day, for the average measurement of the longest diameters of ten of the largest cells was 12.6 microns, in contrast with 9.95 microns, the average at the fifteenth-day stage. These compare in size with the large extraosseous macrophages. Typical uncounterstained cells are shown in figure 15, d. Mitoses in dye-containing cells are present, but are not frequently found.

Again, the presence of numerous hypertrophied and highly phagocytic macrophages in regions where active bone resorption is proceeding is significant. The relationship is similar to that in growing bone.

The same general morphology as heretofore is noted. Even here there are many non-trypanophilic reticulum cells. As before, too, the voluminous thin-walled blood-sinuses are a feature of the areas of disintegrating callus. Bone growth at this stage is limited to the reinforcement of the permanent osseous trabeculae. But few osteoclasts were found. No dye-granules were discovered in them.

Specimen S 13-2 presents essentially the same features in the callus. Here, too, the macrophagic picture is very striking. This specimen shows an interesting condition in the cartilage, which sometimes appears in the callus, as noted at earlier stages, for it is now becoming ossified. In this process the cartilage is modified and hollowed into spaces in which appear blood-sinuses whose environment is characterized by reticulum macrophages, similar to those found in osseous spaces. Osseous tissue is built around the remnants of modified cartilage as in developing cartilage bone. Trabeculae so formed are resorbed like the usual trabeculae of the callus, and the macrophagic tissue is concentrated similarly here. A formation essentially the same as the familiar epiphysial plate, with typical rows of enlarged and modified cells, was observed. The reticular macrophages play the same part here as in callus or growing cartilage bone, for they congregate in regions where tissue is being broken down, as noted by Shipley and Macklin (1916<sup>2</sup>).

This period represents the high-water mark of the macrophagic activity in the callus, and from this time forward there is a gradual diminution in numbers of the cells and in their phagocytic power, as shown by the brightness of the staining. The decline of the macrophagic tissue is coincident with the gradual fall in the rate of bone-erosion, and with the cessation of this process the macrophages of the reticulum revert to the state of the macrophages of ordinary bone marrow. Though retaining in some measure the power of ingesting and storing colloidal dyestuffs, they are then nevertheless relatively small, few, and weakly staining.

Summarizing as to the twentieth-day stage, it may be stated that there is no destruction of tissue in any of the specimens outside of the callus. Extraosseous tissue destruction has ceased coincidently with the falling away of the macrophage concentration. In the callus, however, the same interesting participation of the reticulum macrophages in dealing with the waste products of callus destruction is emphasized. Even in the cleared gross ribs the cells may be described in the callus spaces. It is especially evident from the sections that the greatest concentration of macrophagic tissue is always found in areas of greatest callus destruction and that these cells change their location to accompany the erosive mechanism, thus gaining the position of greatest efficiency for the performance of their duties.

A brief description will suffice for the remaining stages, since the more active processes, outside the bone, have terminated by the twentieth day, and in the callus the stained cells gradually become less and less conspicuous.

## THIRTIETH-DAY STAGE. (S 11-4).

In the *cleared rib* of the thirtieth day the fracture is well healed. The periosteum shows none of the thickening of the earlier stages. Some swelling due to callus is evident at the site of the fracture. There are no staining features of interest. In the *cleared tibia*, too, the bone ends are held together by firm callus.

The *cleared skull* of the thirtieth day has stained but poorly. Trabeculae of callus radiate from the site of the injury. Macrophages are noted within the spaces of the callus, but are not numerous anywhere else.

The *sections* of the *long bone* of this specimen show that there is little trace of the original injury in the soft parts except the abundant scar tissue. As in the last-mentioned stages, macrophages are present in larger numbers than normal in some regions near the bone, often among scattered and small muscle-fibers. They are comparatively small and pale-staining, and are the survivors from earlier and more active periods.

The *callus* of the thirtieth day is very extensive. In texture it is considerably coarser than that of the twentieth day, the trabeculae being stouter and the spaces larger. The area close to the original bone is occupied principally by marrow-tissue. Farther out there is more bone, and the spaces are smaller. Large, thin-walled blood-sinuses are frequent here, and around their walls are great numbers of reticulum macrophages. On the whole, however, the macrophages are much less numerous and striking than at the twentieth day. The principal change in the callus seems to be a strengthening of the permanent trabeculae, with a paring away, here and there, of the temporary bone.

## FIFTY-FIRST (S 17-1), FIFTY-EIGHTH (S 15-1), AND FIFTY-NINTH (S 12-3) DAY STAGES.

The *cleared ribs* of the fifty-first day show an almost normal contour, there being hardly any swelling due to callus and the medullary cavity being quite patent. Some blue staining is present around the site of the fracture, but this is referable to the diffuse staining of the thickened periosteum and scar-tissue at this point. The same faint diffuse blue appears in the fibrous membrane joining the insert to the edge of the trephine opening in the *cleared skull* at this stage and is also present in the fifty-eighth and fifty-ninth day skulls (foot-note, page 8, *c*). There are no macrophages to be discerned in the cleared specimens of these periods. In the *sections* of the fifty-first and fifty-ninth days the staining of the soft parts presents nothing of interest.

The *callus* of the fifty-first day is very compact and thick and can hardly be distinguished from the original bone. Much of the space has been taken up by the new, compact bone. The surviving trabeculae are stout and inclose large spaces containing marrow-tissue. There are in this tissue comparatively few dye-containing cells, and these are relatively small and weakly stained, resembling the macrophages of ordinary marrow. It is evident from the appearance of the bone that little or no osseous resorption is going on. A few giant-cells, containing no dye-granules, are found in the marrow-tissue.

The *callus* of the fifty-ninth day is very dense. The description of it and of the vitally stained cells it contains is similar to that of the fifty-first day.

SIXTIETH (S 11-5) AND SEVENTY-FIRST DAY (S 5-5) STAGES.

The *cleared rib* at 60 days shows little change as compared with that of the fifty-first day. The site of the fracture can hardly be distinguished, so perfect is the repair. In the *cleared skull* of this stage, and that of the seventy-first day, there is the same diffuse staining of the connective-tissue membrane joining the bony fragments and the same absence of macrophages. The *sections* of the sixtieth day show nothing of interest in the soft parts. The *callus* is greater in amount than in the last two specimens described, and hence more time is required for the resorption of the redundant bone. The osseous structure is somewhat less compact and the trabeculae are thinner. There is evidently some osseous resorption still going on. In keeping with this the macrophages are somewhat more numerous and conspicuous than in the fifty-first and fifty-ninth day specimens. These cells, are, however, comparatively small and weakly stained. Bone-building is going on here and there, as the rows of osteoblasts attest.

The *skull sections* show nothing of interest.

Summarizing the stages from the thirtieth to the seventy-first day, it is to be noted that there is no excess of macrophages outside of the bone. No tissue destruction has taken place during this period except in the callus. At the thirtieth day there are some macrophages to be found in the callus-spaces, but sections of the fifty-first and fifty-ninth days show very few of them, so that bone destruction here may be regarded as almost at a standstill. The spaces are large and the remaining trabeculae are much stouter, so that the principal effort has been directed toward reinforcement of the bony trabeculae rather than destruction of them. The occurrence of a few persisting macrophages in the 60-day stage is to be looked upon as a special case where, on account of the excessive amount of callus, the work of destruction was of longer duration.

## DISCUSSION.

From the foregoing account of the appearance of the tissues at the site of fractures and trephine wounds in the vitally-stained rat, during successive stages of healing from the second to the seventy-first day, it is plain that there are two distinct phenomena to be considered. In the earlier stages the most notable feature is the tremendous increase in the number of trypanophil cells which occurs in the injured tissue and exudate of the region and about the damaged surfaces of the bone; at a somewhat later period an equally remarkable development of phagocytic tissue within the spaces of the callus is seen. The discussion falls naturally, then, under two main headings concerned with (1) the phagocytes of the soft parts surrounding the injured bone, or extraosseous macrophages, and (2) the phagocytes of the callus spaces, or intraosseous macrophages.

## EXTRAOSSEOUS MACROPHAGES.

A survey of the specimens from the experimental animals has shown that the macrophage tissue of the soft parts at the wound-site, after a brisk initial rise following the injury, remains at maximum for a few days and then gradually falls away. During the first two or three days these phagocytes may be considered as undergoing mobilization and development. Noticeably increased at the end of 48 hours, they are on the third day very abundant, and so continue during the fourth, fifth, and sixth days, after which their numbers gradually diminish. In the long bones they are not very marked after the fifteenth day, and in the ribs and skulls, since their work is less, they do not persist so long, being reduced almost to normal by the tenth day, or shortly after. This rise and fall is graphically shown in the series of cleared specimens, especially in the ribs, where the area surrounding the wounded bone speedily becomes blue from the accumulated vitally stained cells and (after remaining so for a few days) gradually pales. Sections from the skull and long bones serve to support this finding.

In studying this increased macrophagic tissue from day to day, one is impressed by the close association which it has with the waste material, the result of the trauma. Again and again has it been noted that, where tissue-waste is present in large amount the macrophages are enormously increased, and that as the débris disappears the macrophagic tissue gradually becomes less marked. Indeed, so close is this relationship that the curve tracing out the chronological record of macrophagic intensity roughly parallels that described by the slowly disappearing waste tissue. These facts will be apparent from the following review.

The evidence presented by the *cleared ribs* shows that damaged tissue and exudate, as manifested by diffuse staining of material in the region of the fracture, was, on the second and third days, strongly marked; on the fifth day somewhat less conspicuous; on the sixth day still more reduced; and after this it was not observed.

In comparison, it is to be noted that the macrophages at the fracture-site were somewhat increased over the normal on the second day, and on the third day were very markedly increased, remaining so until the fifth and sixth days, and being reduced on the ninth day. They then gradually diminished. This relation of the macrophages to the demonstrable débris at the fracture-site is graphically set out in table 2, which shows a synchronism, as evidenced by the cleared specimens and sections, between the occurrence of débris (representing dead and dying tissue) and that of macrophages in increased numbers in the vicinity of the débris. Some variations in individual specimens are noted. Thus in the fifteenth and twentieth day stages of the long bones the macrophages are unusually numerous, probably due to the severity of the injury. Again, the skull sections show more débris and as many macrophages on the tenth day as on the sixth; this is due to an infection in this case.

In the *cleared skulls*, as shown in table 2, the findings are very similar. Débris was strongly marked on the second, third, and sixth days, becoming less on the ninth and not appearing after the tenth day. Its removal was thus accomplished in

the first 10 days following the wound. In association with this gradual removal of the waste material there was a marked increase in macrophages of large size and pronounced staining abilities, extending from the sixth to the ninth day; after this the phagocytes became less conspicuous and had reached almost their normal condition on the twelfth day. Though not plainly seen on the third day in the cleared skull they were abundantly present in the sections of this, so that the period of macrophagic activity extends from about the third to the tenth day inclusive. Probably they begin to increase, as in the ribs and long bones, before the third day.

The cleared skulls present one or two minor points of difference as compared with the ribs. The *débris*, as the table shows, disappears somewhat more slowly, and this is associated with a longer duration of the macrophagic tissue. To explain this slowness of absorption we have to consider, in one skull (S 5-1), the element of infection. Again, macrophages are never quite so abundant in the skulls as in the ribs and are perhaps a little slower in their mobilization.

TABLE 2.

	2 days.	3 days.	5 days.	6 days.	9 days.	10 days.	12 days.	13 days.	15 days.	20 days.	30 days.
Cleared ribs:											
Débris.....	+++	+++	++	+	-	.....	.....	-	.....	-	0
Macrophages.....	+	++++	++++	++++	(+++)	.....	.....	(+)	.....	(+)	0
Cleared skull:											
Débris.....	+++	+++	.....	+++	++	+	-	0	.....	-	0
Macrophages.....	0	0	.....	+++	+++	++	(+)	0	.....	(+)	0
Sections, long bones:											
Débris.....	+++	++++	+++	+++	.....	+	-	.....	-	-	-
Macrophages.....	++	++++	++++	++++	.....	+++	(+)	.....	(+++)	(++)	(+)
Sections, skull:											
Débris.....	.....	+++	+++	+	.....	++	0	.....	-	.....	.....
Macrophages.....	.....	++++	++	++	.....	++	0	.....	-	.....	.....

The plus signs indicate roughly the relative amounts of *débris* or macrophagic tissue, as the case may be. Thus + would denote a discernible increase in macrophages as compared with the normal, or the presence of an appreciable amount of *débris*; ++++ would mean an exceptional amount of macrophagic tissue or *débris*, etc. The sign - indicates an absence of *débris* or macrophages, as the case may be. The sign 0 means that no evidence is presented by the specimen. The bracketed signs indicate macrophages which have persisted in regions where formerly tissue was being absorbed. They are comparatively small and weakly staining.

The *sections* of the *long bones* show even more strikingly the same remarkable coexistence of *débris* and abundant macrophagic tissue over a period from the second to the tenth day, with macrophages remaining in the field in reduced amount as far as the thirtieth day.

In the *sections* of the *skull* the same parallelism of *débris* and macrophages is found, extending (in the specimens examined) from the third to the tenth day.

From this summary, the results of which are graphically set out in table 2, it is evident that the occurrence of excess of macrophage-tissue is strikingly synchronous with the presence of waste material. Following rapidly upon the injury, macrophages become excessive in numbers in and around the damaged tissue; these cells are not only larger in size than the usual resting macrophages, but are of

heightened phagocytic power, as revealed by their increased dye-content. It may be inferred, therefore, that this waste material, the result of the trauma, is concerned in some way with the mobilization of the phagocytes and with their accelerated activities; that, in fact, the increase in macrophagic tissue, in volume and in functional efficiency, is a response to the presence of dead or dying tissue.

From table 2 it is also plain that as the dead tissue vanishes, as shown by the tinctorial and histological evidence, the macrophages become less and less conspicuous, their numbers being reduced and their staining weaker. This points to some connection of the phagocytes with the absorption of the débris. More will be said upon this point later.

Another evidence that tissue resorption has been proceeding coincidently with the presence of macrophagic tissue in excess is the thinning and rounding of the ends of the bones surrounded by the phagocytes. This, for instance, is seen at the sixth day (S 11-2) in the cleared rib.

As table 2 shows, some of the phagocytes are found in numbers somewhat above normal after demonstrable débris has disappeared. These are to be looked upon as cells which have persisted in the field after their work was done. They undergo gradual diminution in number and in reaction to the vital dye and are probably to be looked upon as resting rather than as actively functioning.

To anyone familiar with the literature it will be quite obvious that the behavior of these phagocytes, in the reaction following bone injuries, is quite like that which obtains in the repair of any damaged tissue, and thus the problems involved are those common to *inflammation*. These problems have been investigated by various writers, as Maximow (1902, 1906, 1909<sup>2</sup>) and Goldmann (1912); the latter studied the tissues of the vitally stained animal following the application of turpentine and infection with the tubercle bacillus. Tschaschin<sup>1</sup>(1913), too, investigated the reaction of the vitally stained cells in the neighborhood of foreign bodies in the loose connective tissue and in cauterized areas of the liver, spleen, and mesenteric lymph-nodes. Thorough discussions of the various aspects of macrophage behavior under these conditions are to be found in the literature, so that it is here sufficient to refer only to some of the more outstanding points and to emphasize the special application of macrophage function to the repair of bone-wounds.

We have seen that in the rise and fall of excess macrophagic tissue in the areas surrounding bone-wounds a curve is traced. From this three successive segments may be taken to block out periods of macrophage history in which the most outstanding features of the phagocytic tissue are consecutively *development*, *activity*, and *decline*; but it must be recognized that these periods grade insensibly into one another, so that if arbitrary limits be assigned to them there will be of necessity some overlapping. They serve, however, to separate the discussion into convenient subdivisions.

#### DEVELOPMENT.

Of special interest in connection with the development of the macrophage-tissue is the question of *origin* of these phagocytes of the soft parts. Among the possible sources there must be considered the macrophages found normally in the

marrow-cavity and periosteum of bone and in the tissue of the immediate vicinity. When an injury occurs, such as a fracture, it is quite possible to think of these "resting-wandering" cells as undergoing extensive rapid multiplication until the aggregation of phagocytes which is so marked a feature of the early stages of the repair process is produced. But to accomplish this result the proliferation would have to be enormous, and it is noteworthy that but few mitotic figures were to be found among the macrophages.

Again, it is known that endothelial cells can, under the stress of inflammation, take on phagocytic properties, as shown by their reaction to colloidal dyestuffs (MacCurdy and Evans, Tschaschin, and others), and hence it may be assumed that some of the macrophages are recruited from these elements. The reticular cells of bone-marrow, too, are known to be phagocytic, and these may contribute their quota to the sum total of the "polyblasts."

It would be difficult, however, to conclude that any or all of these sources could account for the tremendous local increase in trypanophilic cells, as seen, for instance, on the third day, even providing for the immigration of considerable numbers from adjacent tissues. Again, such an hypothesis would have no place for transitional cells of the type seen in figure 8, and of these there are all grades, from the finished polyblast—large, filled with enormous blue granules, and with a relatively small nucleus—all the way down to what appears to be the parent cell. This is a small mononuclear element resembling a lymphocyte. The parent cells contain no dye-granules, but they soon gain phagocytic ability, as shown by their rapid increase in size and the larger and larger amount of dye which they take up (fig. 8). The transitional cells are particularly abundant in the early stages, and most of all on the third day.

The idea that some at least of the macrophages are really metamorphosed lymphocytes has been steadily gaining in the literature. Even in normal connective tissue, transitional forms may be found linking together the small round amœboid lymphocytes with the clasmatoocytes (Ranvier, Tschaschin) and suggesting the derivation of the latter from the former. The origin of the macrophages of the "tâches laiteuses" in the rabbit is indicated by finding transitional cells connecting them with a small lymphoid element (Tschaschin). Maximow (1907, 1909<sup>1</sup>) has established the relationship of these wandering cells by embryological researches. The same author (1902, 1909<sup>2</sup>) has long maintained that the hypertrophied mononuclear phagocytes or polyblasts of areas of inflammation are largely derived from lymphocytes attracted thither from the tissue-spaces and from the blood-stream, and Tschaschin brings forward evidence to confirm this view—the lymphocytes, it is assumed, rapidly undergoing a metamorphosis, their powers of phagocytosis becoming intensified, as shown by their progressive increase in ability to ingest vital dyes. Tschaschin has illustrated a series of transitional forms in his figure 11, Taf. VII, which strikingly resembles that shown in figure 8. Maximow (1916), according to Downey (1917), has even been able to bring about the development of typical vitally staining polyblasts from lymphocytes in tissue-cultures of lymph-nodes of young



and adult rabbits by the addition of tissue-extracts. Similar views as to the lymphogenic origin of the macrophages are expressed by other authors. In addition to the lymphocytoid cells, most workers recognize the participation, in the formation of the wandering mononuclear phagocytes of inflammation, of the resting-wandering cells of the tissue-spaces, the endothelial cells, and, in the blood-forming organs, the cells of the reticulum, etc.

It is probable, therefore, that while some of the large macrophages of the inflammatory region at the fracture-site come from the reticulum of the marrow and from endothelial cells, and that even more are mustered from the ranks of the resting-wandering cells of the surrounding tissues, yet the vast proportion develop from the small lymphocyte-like cells. Though the lymphocytes of the tissues (histiogenous lymphocytes) doubtless supply some of these, by far the greater proportion probably arises from the lymphocytes of the blood-stream, which have wandered from the vessels into the inflammatory zone (hematogenous lymphocytes).

We may summarize the discussion of the source of the cells by saying that at the beginning of the inflammatory process cells of the types above mentioned are already present in the wound-area, and although these function actively, and even increase in effectiveness, they soon become inadequate to the demands made upon them. Cells from the surrounding tissues, too, may be presumed to wander to the inflammatory region; in these we may recognize representatives of the resting-wandering-cell type and also the closely related "histiogenous" lymphocyte. But even these reinforcements are insufficient for the performance of the work, and the vast bulk of the phagocytes, as we have seen, come by way of the blood-stream.

Thus the macrophages of the inflamed tissue in the vicinity of wounded bone, though derived from cells of diverse morphological type, are yet united by the possession of a common physiological potentiality which manifests itself in a uniform response to a common call to arms. This response consists in the metamorphosis of these cells into enormous and rapacious phagocytes and in the assumption by the latter of an important service in the treatment of the waste products occasioned by tissue-injury. This phagocytic response is graphically demonstrated by the trypanophil reaction. We must postulate a progressive adaptation on the part of the cytoplasm of the mobilized cells until there is produced a mechanism of the highest efficiency in the function of phagocytosis.

It is a striking fact that the working units of this phagocytic tissue, although derived from different sources, resemble one another so closely that the riper forms are indistinguishable. Indeed, this fact has been commented upon by Tschaschin (1913), who, speaking of the resemblance of the "polyblasts" derived from resting-wandering cells to those from the lymphocytes, states (p. 388): "*gegen Ende des zweiten Tages der Entzündung die Polyblasten nach der Quelle ihrer Entstehung nicht mehr unterschieden werden können.*"

#### MOBILIZATION.

Why, it may be asked, do the macrophages congregate at the fracture-site? What is the influence which causes a cell situated near the area of inflammation to

move toward it, or which brings about the dislodgment of a lymphocyte from its ordinary depot into the blood-stream (for we can hardly regard the blood-stream as normally containing sufficient numbers of these cells to supply the requirements without replenishment)? The specific stimulant is undoubtedly the product of tissue breakdown. It may operate through its chemical properties, in which case its action would be described as a chemiotaxis, or its effect may be due to its peculiar physical condition and its influence be more accurately designated as a physicotaxis. Be that as it may, it is certain that the cells themselves are in some way specialized to react to this form of stimulation, not only by moving toward the source of the attraction—in the soft parts of the vicinity by amœboid movement through the tissues, in the blood by movement of lymphocytes from their resting-places into the blood-stream and from this into the inflammatory area—but by developing prodigious phagocytic abilities.

In connection with the hematogenous lymphocytes the attraction must be thought of as acting to get the cells from their depots into the blood-stream, where the attracting influence may be assumed to be circulating. But the latter would not control their course in the blood-current, for in this they float passively until they happen to reach the region of inflammation. Here the circulatory conditions favor their arrest, the blood-current in the dilated capillaries being very slow. Diapedesis ensues, and the embryo phagocytes are thus assembled on the field of their operations. The inflammation-area, as it were, "screens out" the lymphocytes from the blood, just as, in certain types of septic inflammation, the leucocytes which are brought in the blood to the focus of inflammation are sifted out and retained there by the mechanical and other conditions which they encounter.

The interesting fact is noted by Tschaschin and others that the local lymphoid elements of adenoid-tissue do not normally stain with vital dyes and even in inflammation they are very slow in developing phagocytic power. It appears, however, that if these cells gain entrance into the blood-stream they soon become sensitive and react promptly to the stimulus of inflammation by wandering from the vessels and becoming metamorphosed into typical "polyblasts."

#### ACTIVITY.

The method of action of the macrophages has long been the subject of much study on the part of cytologists. There is nothing to suggest that these phagocytes actually break down the tissue. It may be that they secrete some enzyme which assists in this process, but no facts were discovered in support of this view. Their function is concerned with the clearing away of the waste products rather than with tissue solution. In the section describing the observations it has been noted that the cells are not typically in direct contact with the tissue being absorbed. Moreover, they do not seem to operate merely by engulfing fragments of the moribund tissue, although their ability to act in this way on occasion is not questioned; rather, they are concerned with the imbibition of a colloidal solution of the tissue. In this solution they lie, their outer walls bathed with it. The way in which they absorb the material may be inferred from the way in which they are known to take up col-

loidal dyestuffs (Evans and Schulemann, 1914). The ultramicros of such dyestuffs in some way permeate the cell-membrane and are combined in aggregations which soon become visible by the aid of the higher powers of magnification as multitudinous, isolated granules. It appears, too, that often an aggregation of dye-molecules is situated within a vacuole in the cytoplasm. This fact has led some workers to suppose that the cell was attempting to subject the material so housed to a form of digestion in which it would be made useful, or at least harmless, and that its treatment of this colloidal dyestuff was an example of its behavior toward any material in the same physical condition.

The same reasoning may be applied to the interpretation of the behavior of the macrophages in fractures, and we may postulate that the colloidal waste products resulting from the breakdown of the tissue are similarly acted upon within the cell economy. This may be a productive mechanism. It is a well-known fact that certain of the products of protein-splitting, if generally scattered throughout the circulation, will bring about great harm. Now it is quite possible that some at least of the products of proteolysis in fractures and other wounds are of this noxious character, and it may well be that the macrophages are called out to form a barrier against the escape of these materials into the general circulation. There is a stasis of fluids in these regions, and thus the conditions are most favorable for phagocytosis (Downey, 1917). Indeed, in *emergencies* such as the healing of wounds and clearing away of damaged tissue resulting from gross insults, the macrophages may be considered as expressing in an exaggerated form the same function they express every day under normal *routine* conditions of metabolism—as in the breaking-down of red blood-cells, or it may be of the protoplasm of muscle-fibers. Here it is noteworthy that Goldmann (1909) finds these cells especially numerous in the heart—a hard-worked muscular organ. Not all tissues, however, are to be looked upon as producing materials which are dealt with in this way; for instance, nerve-tissue contains few or none of these cells (Goldmann, 1909), and hence the functional waste-products of this tissue may be considered as being treated in some other manner.

#### DECLINE.

As to the fate of the macrophages of the inflammatory area, it has been noted that, shortly after the disappearance of the tissue-waste, the macrophages, in all the specimens, become less and less evident, finally dwindling to their normal numbers (table 2). The examination of the sections throws some light on the final end of the individual cells. As early as the third day we have noted that certain of the phagocytes had a degenerate appearance, and these were more numerous on the fifth day. It seems evident that some of the cells start to degenerate quite early, even at the stage where new macrophages are developing and where the macrophagic tissue is, on the whole, increasing. On the sixth day the degenerate cells were present in enormous numbers, especially in the young scar-tissue, and it is at this time that most of the phagocytes undergo dissolution. After the first week they disappear more slowly, for but few degenerate forms are noted; indeed none was seen after the twelfth day.

The morphology of these degenerate cells has been described in the text (sixth-day stage). It appears that many (probably most) of the phagocytes fall to pieces, their liquefied content of ingested material (which is assumed to be changed in character) being returned to the lymph-spaces, from which it is passed on to the blood-stream, to be excreted or utilized; and any fragments which remain are probably devoured by the tissue-phagocytes.

A few macrophages, however, as has been noted, persist on the field after absorbable material has disappeared. In the fractures of the long bones these were observed in numbers more or less above normal as far as the thirtieth day. They haunt the surviving muscle-fibers and new scar-tissue. Their size and staining ability gradually diminish, and they are quite inferior in these respects to the active phagocytes; their numbers soon fall away to normal.

It is difficult to say, from the evidence, whether or not these cells ever become transformed into fibroblasts, as Goldmann (1912), Maximow (1902, 1906), and Tschaschin (1913) suggest. The typical fibroblasts plainly are quite different from the typical macrophages, and it seems probable that most, if not all, of the scar-tissue arises independently of the macrophages. In the fifth-day stage, for instance, when there is no perceptible diminution in the number of macrophages, there is much new fibrous material. They, however, give place to scar-tissue, and, in a sense, may be said to prepare the way for the scar by assisting in the removal of waste material (Maximow, 1902).

#### INTRAOSSSEOUS MACROPHAGES.

The second outstanding fact brought to light by the study of vitally stained, healing bone-wounds is that the reticulo-endothelial cells of the callus-spaces develop marked phagocytic power coincidently with the appearance of the erosive processes concerned in the enlargement of these spaces; the intensity of this power, too, seems to be roughly proportional to the amount of the callus breakdown. There is, indeed, a most obvious parallelism in the curves tracing the degree of activity of callus destruction, on the one hand, and the degree of phagocytic efficiency of the macrophagic reticulum-tissue on the other, which is consistently maintained throughout the entire history of the callus.

From a review of the findings in the callus up to the sixtieth day it is possible to divide the life of its macrophagic tissue, like that in the degenerate soft parts, into the three phases: *development*, *activity*, and *decline*. Although these phases merge gradually into one another, yet arbitrary limits may be set for them, that of development covering the first nine days, that of activity, roughly, the period from the tenth to the twentieth day inclusive, and that of decline the remaining time. Having made this division, it is a simple matter to recount the most important features of each phase.

#### DEVELOPMENT.

Upon referring to the records it will be noted that the callus rapidly develops, following its first indication on the second day, and by the sixth day is quite well marked. In this callus, spaces filled with cells (which are derived apparently from

the tissue of the Haversian canals of the old bone) early make their appearance. First seen on the third day, they soon expand, the largest spaces naturally occupying the oldest part of the callus, situated, of course, in the vicinity of the original bone.

With the hollowing-out of these spaces numbers of the reticulum cells contained in them gradually acquire the power to ingest and store colloidal dyestuffs. Faintly stained cells were found in the spaces in the third-day stage. This manifestation of phagocytic activity on the part of the cells of the reticulum is intimately associated with the breaking down of tissue consequent upon the excavation of the callus spaces. It is noteworthy that the brightest staining occurs in the cells of the largest spaces, and especially in those at the edge of the old bone—an indication that the greatest phagocytic activity is resident in areas of greatest tissue destruction.

As new spaces open out, trypanophilic cells, at first very weakly stained, appear in them, so that the number of these cells gradually increases, keeping pace with the growing volume of the callus recesses. Cell multiplication is by mitosis, as is proved by finding karyokinetic figures among them, even in dye-containing cells. Also, with advance in age of the reticulum cells, there is usually a slight concomitant progressive increase in their phagocytic potentiality which goes hand in hand with the gradually accelerated callus erosion; for, in the fifth and sixth-day stages, the cells in the older and larger spaces are somewhat more brightly stained than corresponding cells of earlier periods. No noteworthy increase in size of these phagocytes is so far apparent. Thin-walled blood-sinuses of large size appear in the callus-spaces of this period, and are a marked feature. A favorite position of the macrophages is between the walls of these vessels and the osseous plates.

In no area of the callus during this period is the staining phenomenon of the reticulum cells at all comparable with that of the extraosseous macrophages; indeed, the reticulum cells are quite small and inconspicuously stained as compared with the deeply dyed "polyblasts" of degenerating soft tissue, so that it seems more rational to look upon them as cells whose powers are as yet potential rather than actively functioning—as elements, indeed, capable of developing very efficient phagocytic ability on short notice. Though their service in phagocytizing the products of tissue breakdown during this early stage is recognized, it is felt that the amount of colloidal waste resulting from callus erosion can not be very great, since the total callus destruction is as yet small. The main feature of the callus during this phase is construction (rather than destruction) of osseous tissue.

#### ACTIVITY.

The history of the callus during the period from the tenth to the twentieth day inclusive is of the greatest interest. The first part of this interval is characterized by both development and destruction of bone, for as the callus expands in its more outlying regions the older parts are worn away, as shown by the progressively widening spaces. Gradually peripheral growth declines, it being practically nonexistent by the twentieth day, but very active destruction of the trabeculae is maintained throughout. During the first few days the area of most active bone destruction is in the older portions of the callus around the original bone, but as

the tissue here is eroded this active zone shifts outward and at the fifteenth day it occupies the interior of the mass. From here the zone advances, in turn, to the periphery, and the redundant material is cleared away in that region. Certain areas of bone, necessary for the stability of the callus, are conserved, and these are thoroughly reinforced through the application of layer upon layer of bone by the activities of the osteoblasts.

During this period the intraosseous phagocytes, as demonstrated by vital-staining, are exceedingly striking. In regions where obviously bone erosion is proceeding most vigorously they now devour the dyestuff much more greedily and store it in the form of larger and more numerous granules. In their more intense staining they form a striking contrast to the cells of the developmental phase. Thus, in the earlier stages of this period, at the tenth and twelfth days, the largest macrophages are found crowded in the spaces near the original bone; on the fifteenth day they have shifted to the interior of the callus, and on the twentieth day (keeping pace with the outward movement of the zone of most active bone destruction) they have again shifted their ranks to the peripheral regions of the callus, the more central areas containing relatively few of them.

This localization of the demolition-zones is not absolute, for (especially in the later stages) detachments of hypertrophied macrophages may be found in nooks and corners throughout the callus wherever bone is being actively resorbed. Again, in some of the ribs (perhaps because there was less movement of the fragments) the callus was less and its removal was accomplished apparently by a process of paring down from the periphery, the bone becoming more and more slender, as the figures of cleared ribs indicate. It is quite obvious that concentration and hypertrophy of macrophagic tissue are inseparably linked with active bone erosion.

As time goes on, the macrophages of the reticulum undergo, in the areas of active bone destruction, a certain amount of progressive enlargement, the largest cells having an average long diameter of 7.6  $\mu$ , 9.15  $\mu$ , 9.95  $\mu$ , and 12.6  $\mu$  on the tenth, twelfth, fifteenth, and twentieth day respectively. With this hypertrophy there is some increase in the amount of the dyestuff stored, indicating an exaltation of phagocytic power. Mitotic figures in reticulum cells, many of which contained dye, were found throughout this period of activity. A few smaller and less brilliantly stained reticulum cells are found in all parts of the callus tissue.

The *manner of action* of the reticulum macrophages presents some features of interest. There is nothing to support the idea that they carry on, contribute to, or even initiate the *actual process* of callus destruction. Their position in the reticulum—never in actual contact with the bony structure—does not, to say the least, lend support to any such hypothesis; nor is there any evidence pointing to the elaboration, by these cells, of a secretion—such as an acid or a proteolytic enzyme—which would act in the liquefaction of the callus. On the other hand, there is positive evidence of the most convincing kind—the avidity with which these cells ingest colloidal dyestuffs—that they play the rôle of phagocytes; like the polyblasts of degenerating soft tissues, or the reticulo-endothelial cells of developing bone, they ingest the products of tissue breakdown.

The particular tissue-destruction with which the reticulum macrophages have to do is that of callus. In this destructive process we recognize two aspects: (1) the removal of the bone-salts; (2) the removal of the matrix with its contained cells.

It is becoming more and more clear that the process of removal of the bone-salts from the matrix is simply the reverse of that of their deposition in ossification; that we have here to do with the reverse phase of a chemico-physical reaction whose direction is determined solely by the conditions of the immediate environment. Under certain circumstances, apparently centering around definite and well-ordered changes in the local blood-vascular system, there are precipitated from the circulating fluids into a special matrix (elaborated by the activities of definite specialized cells, the osteoblasts) certain insoluble building-materials, the bone-salts. These consist mainly of calcium phosphate and calcium carbonate, their quantitative relations being determined by their relative solubilities in the blood-plasma. There is here simply a cell-controlled calcification (Wells, 1911; Macklin, 1917).

In deossification, or more accurately decalcification, the reverse process is encountered. Changes, particularly in the circulating fluids, cause the bone-salts to be released from the matrix and again taken up into the blood; the matrix and bone-cells remain; the former is liquefied, by means at present obscure. As to the latter, there is ground for the view that they are sometimes left in heaps like drift-wood; that they even coalesce to form giant-cells (Arey, 1917). It is probable that many are disintegrated, to swell the volume of the liquefied waste-products.

That the liquefied bone-salts are phagocytized by the macrophages seems doubtful. They are non-toxic and probably pass off into the circulating blood in a manner the reverse of their incoming. It does seem probable, however, that the phagocytes ingest some, at least, of the products resulting from liquefaction of the protein-content of the callus. Their function would thus be closely allied with that assumed for the wandering macrophages of the degenerating extraosseous tissue. Here, too, it may be postulated that their service is protective; that they guard the organism from the harmful effects of toxic, nitrogen-containing compounds resulting from proteolysis. It is quite possible that only certain of the compounds arising from tissue breakdown are poisonous, and that only these are ingested. As in the case of the polyblasts, it may be assumed that the materials so phagocytized are digested and rendered innocuous—or even useful—within the cytoplasmic laboratory of the macrophages.

It has been noted that the treatment by the macrophages of high-molecular dyestuffs, such as trypan-blue, is an expression of their general behavior toward any material in the same physical condition; and it has been inferred, therefore, by Shipley and Macklin (1916<sup>2</sup>), that the material resulting from the erosion of provisional cartilage and bone, which is phagocytized by the macrophages, is in a finely dispersed state. Such an inference may also be made for the waste products resulting from the erosion of callus or from the breakdown of the soft tissues around the wounded bone.

The *position* of the macrophages of the demolition zones in the loose reticulum of the spaces is favorable to the exercise of their function, for they, like the poly-



blasts of moribund tissue, are bathed by fluid containing colloidal waste products. Especially in the perisinusoidal spaces (where, as has been many times observed, the phagocytes are often thickly crowded) are they well situated to gather in waste products; for here not only do they have access to the materials coming directly from the dissolving tissues, but, owing to the thinness of the vessel-walls and the slowness of the circulation, diffusion of katabolites from the sinuses into the spaces may easily occur, so that these macrophages are in a position to gain some of their pabulum from the blood-stream. In any event, toxic materials in the blood-sinuses are readily extracted by the phagocytic endothelial cells.

The enormous thin-walled *blood-sinuses* of the callus, which have been repeatedly referred to, are a conspicuous feature of areas of active bone-demolition. They arise, as has been shown, through coalescence of smaller vessels. So striking are they on account of their enormous size and great number, as well as because they are invariably present in areas of active callus destruction, that the conclusion is forced upon us that they must play an important part in the breaking-down and removal of the redundant osseous tissue. Certain it is that the rate of flow in these vessels is very slow and that, judging from the thinness and insecurity of their walls, the pressure is very low. It is probable, too, that the  $\text{CO}_2$  tension is high.

It is significant that a very voluminous blood-current of slow speed and low pressure, flowing through a thin-walled channel, enveloped thickly with phagocytes of high-grade efficiency, is so constant a feature of callus resorption, as it is also of the resorption of young developing bone. It suggests that this is part, at least, of the mechanism of bone erosion.

#### DECLINE.

During the third phase, after the twentieth day, osseous resorption gradually ebbs and the destructive and constructive activities of the callus slowly subside. Reinforcement of the permanent trabeculae is the principal industry of the cells, but, especially in the earlier days, evidence of the work of the wrecking-gang is still seen in the clearing away of the few remaining provisional spicules of bone and the trimming of the rough corners. The result is a firm osseous structure formed of material almost indistinguishable from the bone of the original shaft, containing relatively few (but very large) spaces filled with vascular and marrow tissue. This bony formation occupies the fracture-site and thoroughly immobilizes the shaft of the bone.

In keeping with this falling away of destructive processes the macrophages gradually decline in size, concentration, and staining activity, and revert to the character of the ordinary bone-marrow reticulum cells. This does not take place uniformly in all stages, however, for in the specimens of the sixtieth day there was still evidence of bone erosion as well as of bone building, whereas in those of the fifty-first and fifty-ninth days these processes had apparently completely ceased. Involution and degenerate forms, like those of the macrophages of the soft parts, were not found in the callus-spaces.

*Cartilage* was found almost constantly in the callus of the long bones and seems to be associated with movement of the parts during repair, since they were not splinted. It was not found in any of the skulls, where movement was absent. In



the older stages the cartilage underwent changes similar to those of ossification of cartilage in normal skeletal development. A case was found where the cartilage had even taken all the characters of a typical epiphysial plate. Associated with the process of destruction of the cartilage, and of the trabeculae of bone built upon the calcified remnants, macrophages played the same part as in the normal endochondral ossification, as shown by Shipley and Macklin (1916<sup>2</sup>).

*Giant-cells* or "osteoclasts" were found not infrequently in the callus of some of the specimens, and rarely in others. Their numbers seemed to have no relation to the amount of bone destruction; thus none was observed on the tenth day, a few on the twelfth, they were fairly numerous on the fifteenth, but scarce on the twentieth day. In all of these stages there was undoubtedly a great deal of bone erosion progressing. Again, at the same stage but in different specimens, they were inconstant in number; thus in one of the specimens of the six-day stage there were very few, while in the other specimen a fair number was found. In no case was there a large enough number, nor were the cells sufficiently well distributed, to warrant regarding them as the agents of bone erosion. Careful search was made for dye-granules in them, but not a trace was to be found; hence they are not phagocytes of the type of the macrophages. Indeed, no ingested material of any kind could be found in them. Thus the giant-cells of the callus and the underlying old bone in process of erosion are in these respects quite the same as the giant-cells of developing bone described by Shipley and Macklin (1916<sup>2</sup>).

#### COMPARISON OF EXTRAOSSEOUS AND INTRAOSSEOUS MACROPHAGES.

It is of interest to compare briefly the macrophagic cells of the callus with those of degenerating extraosseous tissue. They have many points of difference. The callus cells are fixed and belong to the reticulum tissue, whereas the cells of the soft parts are mostly wandering and are derived from the "resting-wandering cells" of the tissues and from certain lymphocyte-like cells brought in the blood-stream. It is probable, however, that cells originating from the reticulum and endothelium of the bone-marrow of the broken bone-edges contribute to the forces of the extraosseous macrophages in their vicinity. The callus cells develop *in situ*, whereas the cells of the soft parts begin their development probably for the most part outside the zone of their operations; however, both no doubt undergo their hypertrophy mainly at the site of tissue-breakdown. The callus-cells multiply *in situ* by mitosis (typical figures being found in dye-containing cells as early as the sixth day and as late as the twentieth), whereas most of the cells of the soft parts undergo multiplication at their source. The largest of the extraosseous macrophages are of greater size than the phagocytes of the callus, and usually contain more dyestuff, their granules, as a rule, exceeding in magnitude those of the callus-cells.

It seems probable that the demands upon the macrophages of the soft parts are much heavier than those which the callus-phagocytes are called upon to meet, the waste material being much greater in the damaged soft parts than in the resolving callus. Hence the cells of the callus never reach the physical proportions nor the great numbers of the cells of the soft parts. The effort of the extraosseous cells

is sudden, vigorous, and of short duration, their period of maximum efficiency being from the third to the sixth days inclusive; whereas the effort of the intraosseous elements begins later, is more gradual in onset and less violent, and is of longer duration, the period of maximum efficiency of these cells ranging from the tenth to the twentieth days. Both types, however, are apparently stimulated to hypertrophy and functional efficiency by the same type of material—waste resulting from proteolysis; but the call is apparently much more sudden, forceful, and peremptory in the soft parts than in the callus, so that here an expeditionary force of potential macrophages from distant regions must be rushed in. The needs of the callus, on the other hand, in the matter of its resorption, are apparently served adequately by the exaltation of the powers of the resident phagocytes. Both macrophagic types are concerned in like manner in the treatment of dissolved tissue-waste, and they are thus physiologically similar. In both types phagocytic activity has been developed coincidently with the occurrence of tissue breakdown. Both have a reconstructive as well as a scavenger function, for they prepare the ground for scar-tissue or permanent callus, as the case may be. Most of the cells of the soft parts perish *in situ*, while the fate of the callus-cells is obscure; no involution forms, however, were found among them.

Macrophages were found in healing wounds of *membrane-bone*, not only in the surrounding soft parts but in the spaces of the callus itself. Here the cells were of the reticulum type and were related to blood-sinuses as in the long bones. The callus of membrane-bone was rather slower in development and was relatively small in volume, so that the pictures presented by callus in the trephine-wounds of the skull were not so striking and instructive as those in the long bones. They were useful, however, in confirming the findings in the long bones.

It is worthy of emphasis that the method of vital-staining, as applied to *developing callus* on the one hand and to *developing bone* on the other, demonstrates very forcibly that the same cellular elements are at work in the performance of a like task. Macrophages of identical morphological type are found in mass formation in each case, intimately related to areas of bone or cartilage resorption, and for them a common physiological significance is claimed—that of phagocytizing the products of disintegration of provisional cartilage and bone. More than this, there was found absolutely no trace (in the osteoclasts of either callus or developing bone) of any phagocytic activity. Thus the conclusions of Shipley and Macklin (1916<sup>2</sup>) in regard to developing bone are upheld by the findings in callus.

From an examination of all the cleared and sectioned skulls it may be said that no difference can be discerned in the staining reactions consequent upon putting the insert back right side up, or upside down, or inserting living or dead bone from another rat, or even foreign dead bone. Thus the macrophages, as far as could be made out, behave alike toward all these types of insert. In no case was the insert removed, although the edges were trimmed and rounded off; and, although here and there in some of the older skulls there was some slight evidence of erosion in the inserts and in the surrounding bony edges, it is remarkable how little of the insert disappears. The absence of an increased macrophagic tissue,

after the débris has been cleared up, is what would be expected, since so little of the insert is eroded.

A note may here be made as to the condition of the healing areas of skin over the trephined bones. Several of these were cleared and some were sectioned. It was found that macrophages are increased in certain places, especially around the suture holes, in the earlier stages. This was noticed in the skulls of the following days: second (S 18-1), ninth (S 17-2), tenth (S 5-1), and twentieth (S 12-2). Thus the macrophages appear to have a function to perform in the solution of the sutures and probably in the repulsion of infection as well. In the actual scar-tissue the trypanophil phagocytes are few in number. It is of interest to observe that Goldmann (1912, p. 80) noted a similar increase of macrophages in healing skin wounds of the rat.

### CONCLUSIONS.

The following conclusions are based solely upon investigations with the rat. It seems probable, however, that they would hold good in general principle for the other mammals, and no doubt for many of the lower forms.

In the healing of bone-wounds, macrophages, which stain brilliantly with trypan-blue, soon congregate at the site of the injury and become very numerous, hypertrophied, and of increased phagocytic power. They assist in dealing with the tissue-waste resulting from the trauma. These phagocytes are developed principally from the lymphocyte-like cells from the blood-stream, but also from local mononuclear cells with phagocytic potentialities. Most of them ultimately disintegrate *in situ*.

During the structural changes attending the transformation of provisional into permanent callus, trypanophilic macrophages develop in the callus-spaces from the reticulum cells and become numerous, large, and phagocytic. They function in the removal of redundant bony spicules, their particular rôle being concerned with the absorption of the waste products from the breaking down of the matrix. When cartilage is present in the callus they also play a part in its removal. Their action here is thus the same as that of the macrophages of developing bone.

The macrophages of soft parts and bone, though morphologically different, are physiologically similar. They phagocytize the products of proteolysis and segregate the material within their cytoplasm, where they probably subject it to a form of digestion.

Limited numbers of polymorphonuclear leucocytes were encountered among trypanophilic macrophages in areas undergoing repair. No dye-granules were found in them. Physiologically they are distinct from the macrophages.

The osteoclasts of the callus did not show dye-granules. Their numbers did not bear any relation to the apparent amount of bone-destruction which was going on. Osteoblasts, too, contained no dye-granules.

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## DESCRIPTION OF FIGURES.

*B*, original bone.  
*BS*, blood-sinus.  
*C*, callus.

*M*, macrophage.  
*MC*, marrow-cavity.  
*Mus.*, muscle.

*N*, nerve.  
*P*, periosteum.  
*S*, scar-tissue.

### PLATE 1.

- FIG. 1. Portion of normal rib from rat vitally stained with trypan-blue. The black dots represent macrophages normally present in the marrow-cavity, periosteum, and tissue around the bone.  $\times 27$ . This and the next five figures are free-hand drawings from gross cleared preparations under the binocular microscope. The macrophages are represented somewhat larger than they actually are, for the sake of plainness.
- FIG. 2. Fractured rib of rat on second day of repair. Note the debris around the ends of bone and the slight increase in macrophages as compared with figure 1.  $\times 27$ .
- FIG. 3. Fractured rib of rat on third day of repair. Great increase in macrophages is seen, with swelling due to young callus. Diffusely stained debris present.  $\times 27$ .
- FIG. 4. Fractured rib of rat on ninth day of repair. Macrophages somewhat less numerous. Callus much more dense.  $\times 27$ .
- FIG. 5. Fractured rib of rat on twentieth day of repair. The contour of bone is almost normal, the medullary cavity is being restored, and macrophages are but little in excess over the normal.  $\times 27$ .

### PLATE 2.

- FIG. 6. Trephined skull of rat on ninth day of repair. A bone-disk has been inserted on the left side, while the right has been left open. Note the crowds of macrophages in the open spaces and around the bone edges. Here and there are areas almost free from phagocytes; these are patches of scar tissue. Early callus spicules line the bone edges.  $\times 19$ .
- FIG. 7. Photomicrograph of area near broken end of long bone from rat on third day of repair. The edge of bone (*B*) and the fragments of dead tissue are stained blue. Myriads of macrophages (*M*) are found throughout the figure.  $\times 40$ .
- FIG. 8. A few cells selected from specimen from which figure 7 was made, showing the development of the large macrophages from the small lymphocyte-like cells. As a rule, more and more dyestuff is taken up with increase in size of the cell. Besides the dye the cells also contain the tissue-waste which they have phagocytized. Free-hand drawing.  $\times 1,000$ .

### PLATE 3.

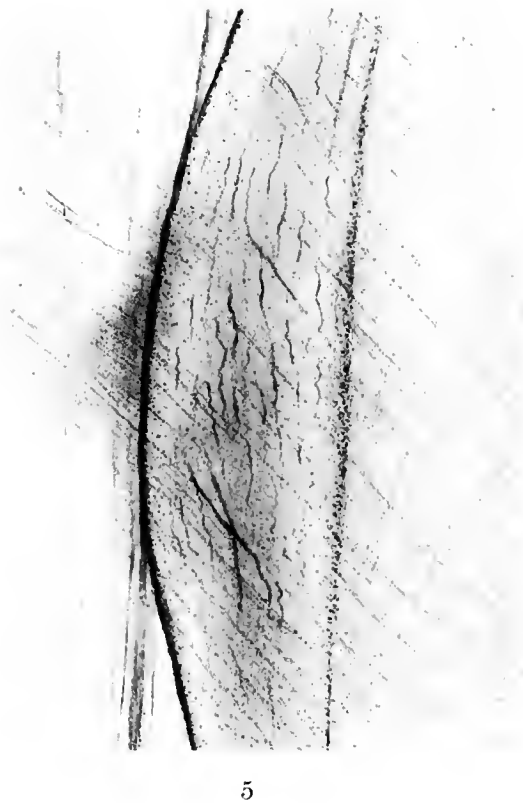
- FIG. 9. Photomicrographs from a cleared and uncounterstained section of the sixth-day stage in fracture-repair. Exactly the same field is seen in the two pictures, but that on the left received a much longer exposure, so that practically the only objects seen in it are the macrophages. These are larger and more thickly distributed in the central and lower right regions. Here the greatest amount of diffusely stained tissue-waste is present, as is seen by the denser staining in this region in the right-hand picture. The field is from an area of degenerating muscle. Above and to the right is scar-tissue (*S*), while below and to the left the muscle is beginning to deteriorate. In the central and lower right areas the muscle is degenerate, and here the macrophages (*M*) are largest and most numerous.  $\times 50$ .
- FIG. 10. High-power drawing (camera lucida) of area of degenerating muscle from a region similar to that shown in figure 9 (*M*). Fragmented muscle-fibers are seen, together with young fibroblasts, polymorphonuclear leucocytes, and macrophages. Three small lymphocytoid cells appear. Some of the macrophages contain more blue dye than others; the latter are usually stuffed full of phagocytized material. One small macrophage has engulfed a polymorph.  $\times 1,000$ .
- FIG. 11. A few degenerate macrophages from an area of scar-tissue, similar to that shown in figure 9 (*S*). They are of different sizes. Some appear vacuolate and ragged; others are mere fragments. They contain comparatively little dye. Some young fibroblasts are shown.  $\times 1,000$ .
- FIG. 12. A portion of a callus-space from the sixth-day stage. A thin-walled blood-sinus (*BS*) is conspicuous, and between its walls and the bone (*C*) are situated reticulum cells, some of which contain dyestuff (*M*) and are thus phagocytic.  $\times 1,000$ .
- FIG. 13. Three reticulum phagocytes from the callus of the sixth-day stage, showing mitosis in dye-containing cells. There are two metaphases and an anaphase.  $\times 1,000$ .

### PLATE 4.

- FIG. 14. Old bone (*B*) and callus from the tibia of the tenth day. Camera-lucida drawing, from cleared section. The spaces near the original bone are filled with the largest and brightest macrophages (*M*). Under high magnification these large phagocytes are shown in figure 15 *a*. Here, too, there are blood-sinuses. Farther out the macrophages are less conspicuous and are absent at the periphery.  $\times 80$ .

## PLATE 4—Continued.

- FIG. 15. The groups *a*, *b*, *c*, and *d* (see plates) were drawn with the aid of the camera lucida from typical large phagocytic reticulum cells selected from the callus spaces of the tenth, twelfth, fifteenth and twentieth days respectively. The sections were cleared without counterstaining and the dye-granule contour only is represented. In the interior of the cell the nucleus is indicated by the clear space. Only mature phagocytes were selected in each stage. They resemble one another closely and show a certain amount of increase in size with age of callus. In each case the drawings were made from cells situated in areas where active osseous resorption was going on.  $\times 1,000$ .
- FIG. 16. A space in callus, showing developing macrophages of the reticulum (*M*). Camera-lucida drawing from peripheral region of callus at the twelfth day of repair in long bone. Some of the cells are clear, while others contain varying amounts of dyestuff. The granules are at first small, but gradually increase in size and number. Large intercellular spaces will be noticed. Blood vessels are not shown in this figure, but in the older spaces at this stage they were large and abundant.  $\times 1,000$ .
- FIG. 17. Drawing made from a tracing from a photomicrograph of the twentieth-day stage of repair. Section cleared without counterstaining. In the lower left corner the old bone and marrow cavity is seen. Around it the callus is arranged, and in many of its spaces there are crowds of black granules, representing macrophages of the reticulum.  $\times 50$ .
- FIG. 18. A small field from the callus of figure 17, much enlarged. Drawing traced from a photomicrograph. Throngs of macrophages (*M*), heavily loaded with dye-granules, present themselves between the osseous trabeculae (*C*). These cells are seen more highly magnified in figure 15*d*.  $\times 190$ .







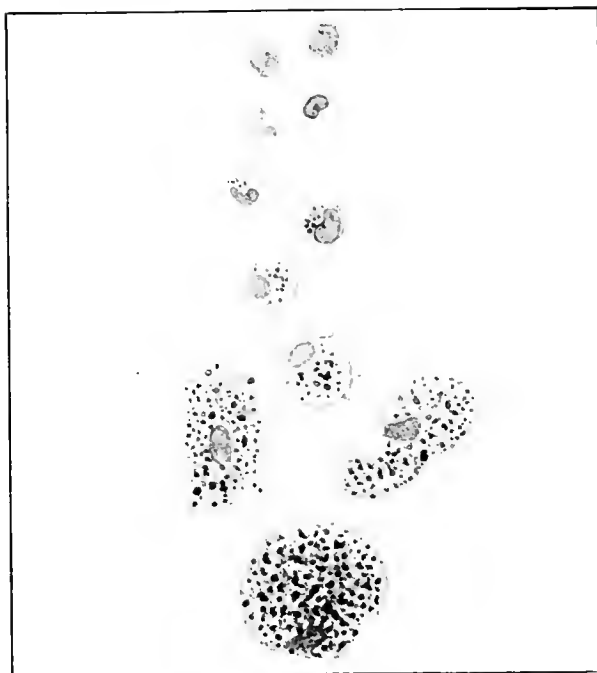


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B

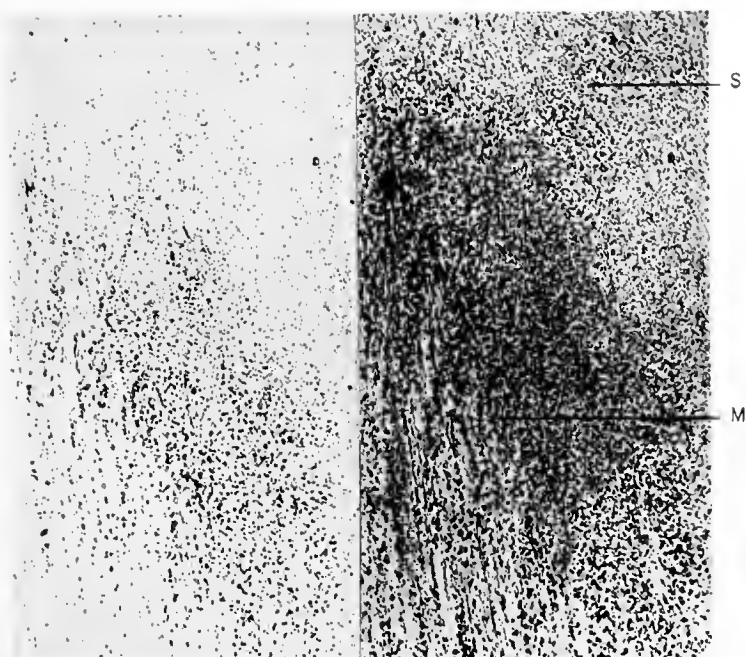
M



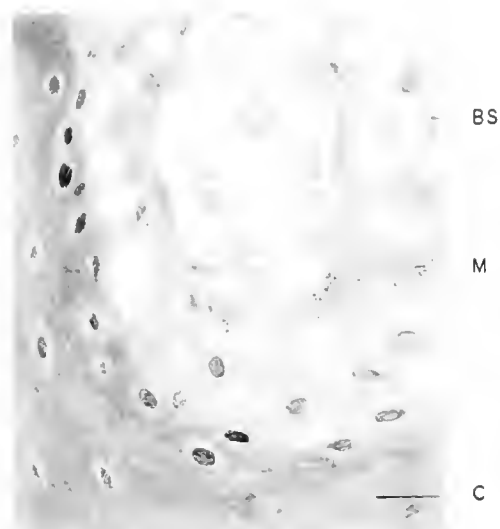
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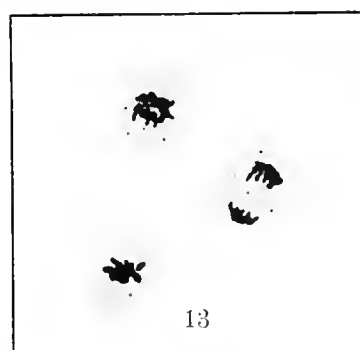




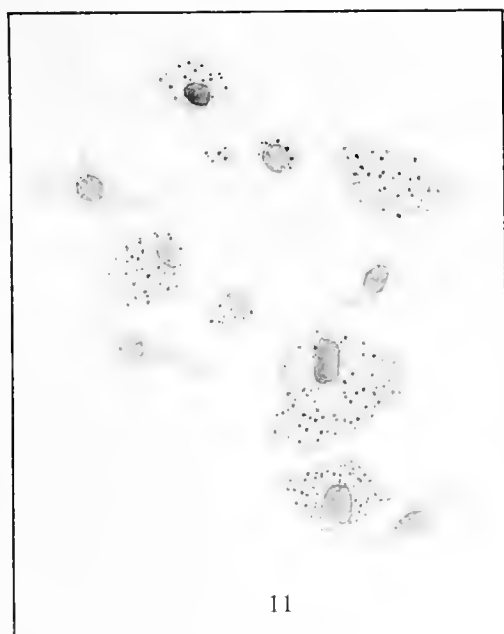
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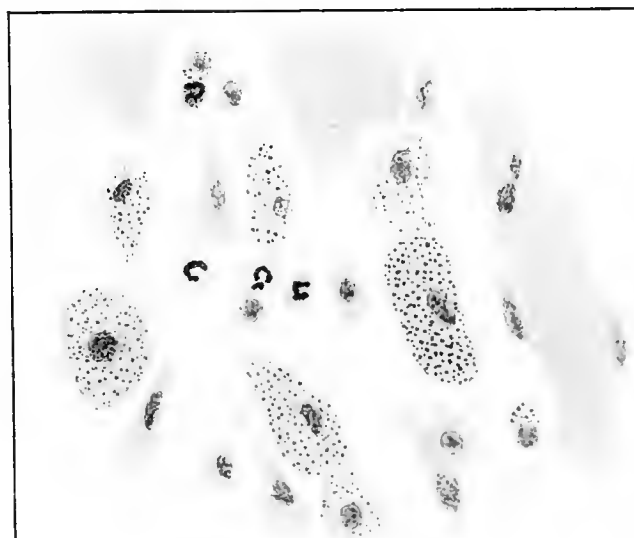
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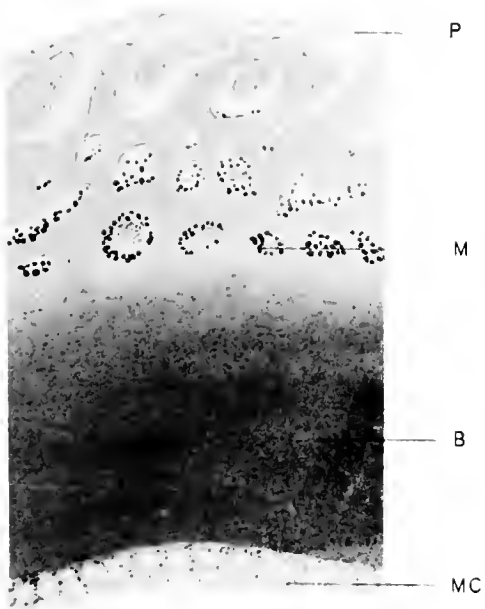


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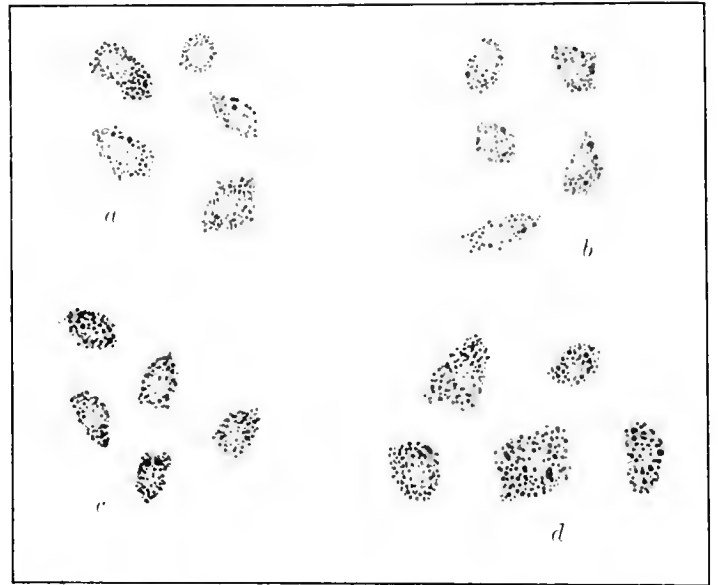


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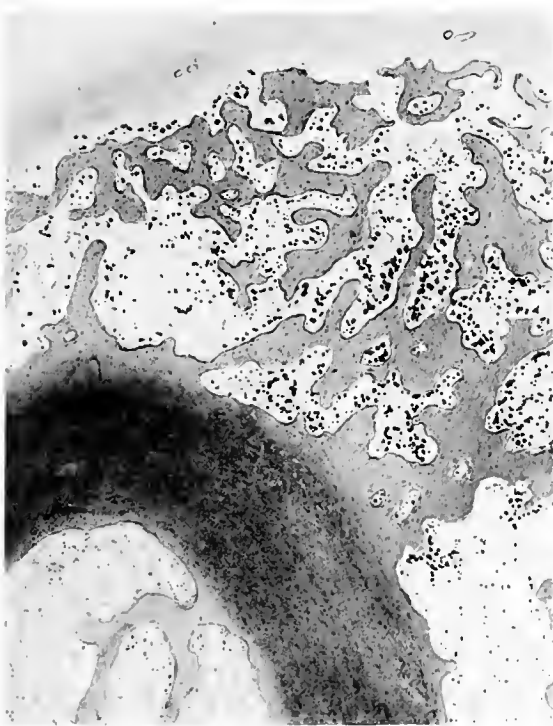




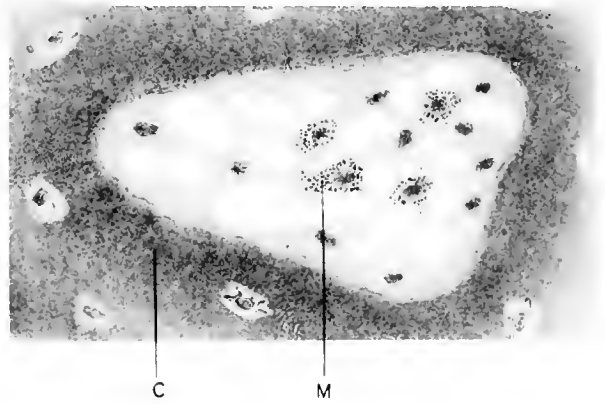
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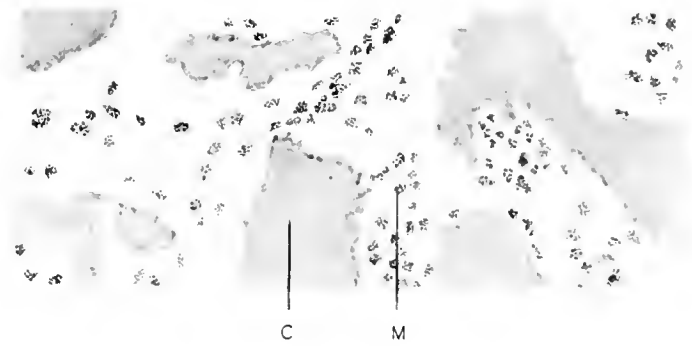
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CONTRIBUTIONS TO EMBRYOLOGY, No. 28.

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CYTOPLASMIC STRUCTURES IN THE SEMINAL EPITHELIUM  
OF THE OPOSSUM.

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With two plates and five text-figures.

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## CONTENTS.

	PAGE.
Material.....	49
Technique.....	50
Short survey of the process of spermatogenesis.....	51
First period.....	55
Second period.....	56
Third period.....	59
Fourth period.....	60
Chondriosomes.....	62
Sertoli cells.....	63
Spermatogonia.....	63
Spermatocytes.....	63
Spermiogenesis.....	64
First period.....	64
Second period.....	65
Third period.....	65
Fourth period.....	67
Discussion.....	69
The apparatus of Golgi.....	73
Discussion of apparatus of Golgi.....	75
Bibliography.....	82
Explanation of figures.....	84



# CYTOPLASMIC STRUCTURES IN THE SEMINAL EPITHELIUM OF THE OPOSSUM.

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BY J. DUESBERG.

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## MATERIAL.

The object of the present investigation was the study of the chondriosomes and of Golgi's apparatus in the seminal epithelium of the opossum (*Didelphys virginiana*), the material consisting of nine animals. Five of these were full-grown; a sixth exhibited all stages of spermiogenesis but the very last—*i. e.*, the formation of the spiral filament at the expense of the chondriosomes; no spermatozoa were found in the epididymis. The three remaining animals were in a much less advanced stage of development. Whether spermatogenesis in the opossum is taking place throughout the year, or whether the testicle becomes active only before the period of copulation, could not be ascertained, as all the material was procured between August and January. It should be stated, however, that the animals which showed only early stages of spermatogenesis were distinctly smaller than those which had spermatozoa and were probably under one year old.

In the adult opossum, at least at the time of year when these animals were collected, the testicle exhibits all the characteristic features of a typical mammalian testicle, particularly that striking regularity in the evolution of the germ-cells which was first revealed in the rat. The images are, however, not exactly superposable, as the duration of the different stages is not the same in both species. I found, for instance, that in the opossum the migration of the ring (which in the rat takes place at the time the cells of the subjacent layer have reached the end of the period of maturation) occurs somewhat later, as the period of maturation is over and the spermatids have already differentiated as far as those represented in figures 17 or 32.

I was impressed by the large amount of fat—that is, such fat as blackens with osmic acid—usually present in the seminal epithelium. Fat appears in the seminal cells at the end of the growth period and a number of small fat droplets are constantly found in the dividing spermatocytes (fig. 6) and in the young spermatids (fig. 7). It then disappears very quickly and none is to be found after that stage. The bulk of the fat present in the seminal epithelium is contained in the Sertoli cells, and it is here that variations may occur. This fat is apparently a product of the degeneration of the cytoplasm (Regaud's "corps résiduel") cast off by the spermatid at the end of its evolution.

## TECHNIQUE.

Fragments of testicle were fixed in the following fluids: Hermann's, acetic sublimate, Ramon y Cajal's mixture of formalin and uranium nitrate, Bouin's, Flemming's, saturated sublimate, Altmann's, Meves's, Benda's, and Regaud's. Fragments of the epididymis were fixed in the latter two reagents. Smears of spermatozoa were fixed either by the action of vapors of 1 per cent osmic acid in a moist chamber for 20 to 30 minutes, or by immersion for 10 to 30 minutes in Regaud's mixture, then for 24 hours in 3 per cent bichromate. Two adults were sacrificed for the purpose of studying the living cells, and were injected with a solution of janus-green ( $\frac{1}{10000}$ ) in 0.85 per cent salt solution. I am greatly indebted to Professor E. V. Cowdry for his help in carrying out these experiments.

Of the above-mentioned reagents Benda's, Meves's, Regaud's and Altmann's fluids were used for the purpose of fixing the chondriosomes. Perhaps the best preparations were obtained from Regaud's material. Regaud's fluid, however, is an exceedingly one-sided reagent. While it often fixes the chondriosomes perfectly well, other structures, such as centrioles, idiozome, axial filament, or "tingierbare Körner" are scarcely visible, if at all; the cell limits disappear and one no longer wonders at Regaud's peculiar conception, according to which the spermatogonia have no cell-bodies of their own (1908). As to the nucleus, there is no possibility of studying it during the growth or maturation period. In the later stages, however, especially with a good nuclear stain, such as methyl green, the same material proves very valuable. The worst feature in the action of Regaud's fluid is the dislocation it produces in the seminal epithelium, a dislocation due (in part at least) to the fact that the fat is not fixed and is consequently dissolved during subsequent manipulations.

A number of nuclear stains and (for the study of the chondriosomes) iron hematoxylin, Benda's and Altmann's method, and acid fuchsin-methyl green were used. Excellent preparations were obtained with the latter method, especially after fixation in Regaud's fluid. As it happened that my material would keep too much methyl green, I found it very useful to bring back the slides from the 95 per cent alcohol into distilled water, a procedure which eliminated immediately the superfluous green and improved the preparations greatly, then back to 95 per cent alcohol, and so forth. In order to bring into view the apparatus of Golgi, I used, with the same success as previously (1914), Ramon y Cajal's mixture of formalin and uranium nitrate. The best preparations were obtained by leaving the pieces in the fixative for 9 hours, in the silver nitrate 37 hours, and in the developer 14 hours. While undoubtedly this method almost unfailingly impregnates the apparatus of Golgi, it is nevertheless a capricious one, inasmuch as it is liable to bring into evidence at the same time granulations, most probably mitochondria (fig. 26), fibrils in the connective tissue, cell limits, and eventually other structures difficult to identify.

Instead of other complicated procedures, at the suggestion of Professor Cowdry I used the following method in the treatment of the sections: The slides were first

immersed on 0.1 per cent gold chloride for 2 to 3 hours; then in hyposulphite for 20 to 30 minutes. The results were excellent. Probably any nuclear dye could be used as counter stain. I resorted to Ehrlich's hematoxylin, to safranin, and to methyl green (Cowdry, 1916), which in the concentration of 0.5 per cent, applied for 30 to 60 seconds, gives a very sharp contrast with the black color of the apparatus and the pale background.

#### SHORT SURVEY OF THE PROCESS OF SPERMATOGENESIS.

An accurate and profitable study of the chondriosomes and of Golgi's apparatus in the testicle can not be made without an intimate knowledge of the whole process of spermatogenesis, and especially of its last phase—spermiogenesis. In this connection von Korff's researches (1902) on the spermiogenesis of another marsupial (*Phalangista vulpina*) proved to be of great help. The process in *Phalangista* is so similar to that in *Didelphys* that it appears necessary to give a summary of von Korff's paper.

Since the work of Meves on the spermiogenesis of the guinea-pig, this phase in the evolution of the seminal cells has been usually divided into four periods: The first period extends to the formation of the so-called "Schwanzmanschette," which, following Oliver's example (1913), I shall call the *caudal tube*; the second ends with the disappearance of the same formation; the third extends to the time of the expulsion of the spermatozoa into the lumen of the seminiferous tubules and of the elimination of the major part of the cytoplasm; the fourth period includes such changes as may take place subsequently—changes which, in many cases, are of minor importance. In *Phalangista* von Korff found that, as the caudal tube appears and disappears suddenly, it is better to use as a basis for the subdivision of spermiogenesis the modifications of the centrioles which coincide with the apparition and disparition of the caudal tube; *i. e.*, their close relationship with the nucleus at the end of the first period and the beginning migration of the centriolar ring which marks the end of the second period. It appeared also expedient to subdivide the second period according to the modifications of the nucleus: First, the spherical nucleus is transformed into an egg-shaped body whose long axis is perpendicular to the axial filament; later it assumes its definite shape.

The young spermatid of *Phalangista* contains two granular centrioles, located first at the periphery of the cell, later moving toward one pole of the nucleus, while the idiozome moves toward the other pole. The distal centriole carries a thin filament and flattens out somewhat at the end of the first period. The modifications of the idiozome are less complicated in *Phalangista* than in other mammals. Instead of the numerous granules, each located in a vacuole as noted in other species (the guinea-pig for instance), a single vacuole without a granule is formed. This attains a considerable size and applies itself to the nucleus, while the remainder of the idiozome is eliminated. Von Korff mentions also a chromatoid body which later falls to pieces and is probably cast off at the end of the process.

The beginning of the second period is marked by the sudden appearance of the caudal tube. The nucleus flattens out and later assumes its most characteristic

shape. The proximal centriole is inserted on the nucleus and very soon it becomes apparent that this insertion is not directly in the center of the nucleus, but toward one extremity ("*laterale Insertion*"). The distal centriole assumes the form of a disc, the middle portion of which, carrying the axial filament, later breaks off, leaving a ring and a small granule (*distaler Centralkörperknopf*). When the head has assumed its definite shape one finds this granule connected with the proximal centriole by a thin filament. Both are located in the notch of the head. The axial filament is very thin in the region of the future middle piece, while posteriorly it suddenly becomes much thicker. This part, which corresponds to the future main piece, exhibits a peculiar structure, a cross striation. The protoplasmic body has elongated and (contrary to what is found in many other mammals) extends farther back than the middle piece.

With the sudden disappearance of the caudal tube the third period begins. Most remarkable is the fate of the headcap, which is left by the spermatozoon in the protoplasm of the Sertoli cell. The centriolar ring migrates to the end of the middle piece, while the proximal centriole breaks into two granules connected by a thin filament with each other and with the granular part of the distal centriole. The chondriosomes dispose themselves around the axial filament. Finally, most of the protoplasm, with the so-called von Ebner's "tingierbare Körner," is eliminated.

A striking change occurs during the fourth period, a change in the position of the head in relation to the tail. At first the axis of the head was at right angles with that of the tail, but in spermatozoa collected in the epididymis the head is found in the same long axis with the tail.

From this short summary it appears that the process is similar to what is observed in other mammals, the modifications of the centrioles, compared with those which occur in the guinea-pig, belonging to a rather simple type. The most striking feature of the process is the elimination of the headcap from the spermatozoon.<sup>1</sup>

While it appeared necessary to give a summary of von Korff's paper on account of the marked resemblance between his description and my own findings, and because of the close relationship between the species investigated, it is not my intention to review again the whole literature relating to spermiogenesis in mammals. I refer the reader to my paper of 1908, and will limit myself to an account of two papers which have appeared since and which are concerned with the process of spermiogenesis, or certain phases of it in mammals—one by Oliver (1913) on spermiogenesis in the fur seal, the other by Stockard and Papanicolaou (1916) on the modifications of the idiozome in the guinea-pig.<sup>2</sup>

Oliver's conclusions agree in the main with those of Meves (1899), von Korff (1902), and the author (1908). There is, however, one point of difference. While Oliver admits, rightly in my opinion, the formation of the caudal tube at the expense of cytoplasmic material—here, as in the guinea-pig (Meves, 1899) its formation from filaments is quite apparent—in opposition to Meves, myself, and a

<sup>1</sup> A paper by Benda (1906) on spermatogenesis in marsupials was not available in this country. I can therefore only quote from my own former references to it (1908, 1912).

<sup>2</sup> E. Allen's "Studies on cell-division in the albino rat III" (Journ. of Morph., vol. 31, 1918), appeared too late for discussion here, but I should like to warn the reader that practically wherever I am quoted in that paper such quotations are inaccurate.

number of other authors, he believes that the caudal tube eventually intervenes in the constitution of the middle piece. Since in the guinea-pig, the degeneration of the caudal tube is obvious, Oliver's description would lead one to believe that the same structure can undergo a different fate in different mammals. I must say, however, that I am not convinced. Oliver describes the process as follows: The caudal tube breaks off the nucleus just before the centriolar ring begins to migrate, then becomes increasingly narrower, surrounding more and more closely the axial filament. At the same time "the cell membrane covering the caudal tube has fused indistinguishably with the latter." The question which immediately suggests itself to me, but which, if Oliver's description be correct, I am unable to answer, is, where are the chondriosomes? These bodies are never, to my knowledge, found within the caudal tube, nor can they be located between the caudal tube and the cell boundaries, according to the quoted description. I would add also that the separation of the caudal tube from the nucleus appears to me, from Oliver's drawings, to be an accidental rather than a normal occurrence; and that between figure 32, in which the caudal tube is still far from being fused with the axial filament, and the next figure there is an important, unfilled gap.

Stockard and Papanicolaou have given a description of the fate of the idiozome (they spell it *idiosome*, after Regaud's proposal, 1910) in the guinea-pig. The present summary is made from their communication at the meeting of the Anatomical Society (1916) and from the abstract published in the bibliographic cards issued by the Wistar Institute (No. 155). The idiosome of the primary spermatocytes consists of two spheres, one inclosed within the other. During the process of division the outer sphere (idioectosome) breaks into irregular pieces, while the inner one (idioendosome) forms a great number of granules (idiogranulomes). The pieces of the idioectosome and the idiogranulomes flow together in each of the secondary spermatocytes and form a new idiosome consisting of a spherical idioectosome which contains a number of idiogranulomes. During the division of the secondary spermatocytes the idioectosome breaks up again, and its pieces are dispersed in the protoplasm with the idiogranulomes. This process permits a uniform distribution of the very important idiosomatic material during the division. In the spermatids a new idiosome is reorganized, having a new idioectosomatic sphere inclosing idiogranulomes, each granulome being surrounded by a small vacuole (idiogranulotheca). The idiogranulomes and the idiogranulotheca flow together to form a large spherical body (idiosphærosome) inclosed within a large vacuole (idiosphærotheca). The idiosphærosome differentiates into an "upper" cap—the idioalypptosome, and a "lower" body—the idiocryptosome; "the idiosphærosome secreting as soon as formed on its surface, furthest from the nucleus, a new substance—the idioalypptosome" (1916). The idioectosome (called also idiophthartosome) is eliminated with the protoplasmic remains. The idiocryptosome and the idioalypptosome persist in the spermatozoon as two caps, one beneath the other and inclosed within the spermioalyptra or idioalypthrotheca, which is the transformed idiosphærotheca.

The new and interesting part of this paper is the description of the behavior of the idioendosome and its idiogranulomes (or better, perhaps, idiogranulosomes) during mitosis, although it must be stated that the persistence of the idiogranulomes in the dividing spermatocyte has already been reported by Niessing (1902), who represents them (fig. 12) in the prophase of the second division. Nothing was known, however, of the behavior of these bodies in later stages; that is to say, of their repartition between the daughter-cells. As to the constituents of the idiozome in the first spermatocytes, Niessing (1897) had already described it in the same species as formed by two layers. Numerous other authors have recognized the same structure in other species and applied to it various names. Stockard and Papanicolaou's *idioectosome* is nothing but Platner's *Nebenkernstäbchen*, Heidenhain's *Centralkapseln*, Ballowitz's *Centrophormien*, Perroncito's *dyciosomes*, Terni's *formazioni periidiazomiche*, etc. This point will be discussed later. A process similar to the behavior of the idioectosome during mitosis has been described by Platner and called by Perroncito *dyciocinesis*. Little is added to what we already knew of the fate of the idiozome during spermiogenesis. The idiogranulomes (Moore's archosomes, 1894), their idiogranulotheca (Moore's archoplasmic vesicles), the fusion of these bodies into one single vacuole and one single granule (which is the *acrosome* or *Spitzenkörper*), the presence within the acrosome of another granule, their relations with the nucleus, and the fate of the vacuole which ultimately forms the "Kopfkappe" or head cap (Stockard and Papanicolaou's *spermio-calyptra* or *idiocalypthrotheca*), have been described in detail for the guinea-pig by Meves (1899). Moore and Walker (1906) also have described two parts in the acrosome, and call the outer part "intermediate substance." None of these authors, however, have been able to distinguish these parts after the first period, but Niessing (1897) was able to follow them until advanced stages. They correspond most probably to the different zones which I have represented (1910) in figures 62, 67, and 68.

*A priori*, it appears that the nomenclature of Stockard and Papanicolaou is by far too complicated to be accepted. From the preceding considerations one may further conclude that it is also unnecessary since most of the things thus designated have been described before, and even prejudicial since some of them (the head cap and acrosome for instance) already have names to which but slight objection can be raised and which are generally accepted.

Furthermore, the description of these authors is inaccurate in several respects: (1) They describe the appearance of the granules only at the prophase of the first division, whereas these exist prior to that time (see Meves, 1899, fig. 2, and perhaps also Niessing, 1897). (2) Similarly, the vacuoles, which they find only in the spermatids, are already present in the second spermatocytes, according to Meves (1899, fig. 3) and to Moore and Walker (1906, figs. 27 and 28), and indeed the last-named authors describe them in the first prophase (fig. 21). (3) The fragmentation of the idiozome does not necessarily take place before the first metaphase, as I found in 1910 (fig. 54), and again recently in preparations of the testicle of the guinea-pig with Cajal's method. (4) The outer substance of the acrosome can not be consid-

ered as a secretion of this last body "on its surface furthest from the nucleus," as the two parts are already differentiated in the perfectly spherical acrosome before its connection with the nucleus. I refer to Meves's figure 9, and to Moore and Walker's figures 39, 40, and 41, and would call attention to the fact that Meves insists (pages 344 and 389) that this is not an appearance due to extracting the stain more or less, as both parts can be distinguished in unstained preparations. Finally, concerning the vacuolar structure of the outer part of the acrosome, I consider the point of little importance, as its appearance varies considerably according to the fixing reagents and type of stain used. (See for example figs. 61-68, Duesberg 1910.)

I come now to my own observations on the opossum and hasten to declare that I have little to say here in regard to the spermatogonia and the spermatocytes. Their nuclei I did not study, and their cytoplasmic constituents will be described in the following chapters. Concerning the first spermatocytes I would only mention that again, as in the rat, guinea-pig, and cat (Duesberg, 1910, p. 66), the growth period can be divided into two phases:

"Dont l'une va jusqu'à la formation des grosses travées chromatiques, tandis que l'autre est postérieure à ce stade. Au cours de la première phase, le spermatocyte de premier ordre est très petit et s'accroît relativement peu. Pendant la seconde, protoplasme et noyau augmentent fortement de volume, l'augmentation de volume du noyau étant particulièrement remarquable chez le rat. Il y a donc ici quelque chose de comparable à ce qui se passe au cours de la période d'accroissement de la cellule sexuelle femelle, la seconde phase correspondant à la période dite du grand accroissement (Grégoire, 1908) de l'ovule."

Mention should be made also of the idiozome as it appears after the action of reagents containing osmic acid. This body consists of two parts, an outer, darkly-staining shell, and a lighter medullar substance. In the spermatogonia and in the young spermatocytes it is usually flattened against the nucleus, and in preparations in which the chondriosomes are preserved it is often entirely covered by these bodies (figs. 2 and 4). In the older spermatocytes the idiozome rounds out and then it is clearly apparent that the outer shell is missing opposite the nucleus (fig. 5). In the dividing spermatocytes it can be occasionally recognized as late as the metaphase, after which it falls to pieces. What becomes of these pieces is difficult to determine without the application of special methods. (See chapter on apparatus of Golgi).

Concerning spermiogenesis I would say that no attempt was made to describe the process in every detail. The study was undertaken in order to build on a safe foundation my researches on the chondriosomes and the apparatus of Golgi. I would also add that I was handicapped, especially in the study of the centrioles, by the lack of good iron-hematoxylin preparations. Enough could be elucidated, however, to make it appear worth publishing. As to the subdivision of the process, my experience was similar to that of von Korff, and like him I have to adopt the modifications of the centrioles as a basis.

#### FIRST PERIOD.

The young spermatid (fig. 7) contains, in addition to the chondriosomes with which I shall deal later, a spherical nucleus, an idiozome, and two granular centrioles



located at the periphery of the cell and perpendicular to its surface, with a thin filament in connection with the distal centriole.<sup>1</sup> No chromatoid body was observed. A number of fat droplets are invariably found in one heap quite near the periphery of the spermatid; these very soon disappear (fig. 8). Their constant location at the periphery, as well as a special lighter aspect of the protoplasm in that region, suggests the possibility of an elimination in bulk. This process was not observed, however, and consequently one may just as well admit that they are simply digested. After a short period of growth the nucleus gradually diminishes in size while the condensation of the chromatin is taking place. There is no change in the form of the nucleus, but there is a change in its location. At the end of the first period it has moved to the periphery of the cell opposite the centrioles (fig. 9).

The idiozome is composed of the same constituent parts as described for the spermatocytes. Then, as in *Phalangista* (von Korff) a single vacuole appears within the medullar part (fig. 8); this increases to rather considerable dimensions, and coming in contact with the nucleus apparently exerts some pressure upon the nuclear membrane, as the latter shows a marked degree of flattening (figs. A, 8 and 17). The nucleus, however, resumes its spherical shape as the zone of contact with the vacuole becomes larger. Meanwhile, the cortical part of the idiozome has been detached; we find it at the end of the first period loose in the protoplasmic lobe. The head cap now extends over the anterior third of the nucleus, or thereabouts, and a small granule, a sort of acrosome, can be seen, although no trace of it was visible in the earlier stages (fig. 9). The centrioles, whose position, from the very beginning of the process, determines what it is customary to call the posterior pole of the spermatid, migrate toward the nucleus, and consequently the axial filament increases in length. The proximal centriole comes in close contact with the nuclear membrane and thus is often very difficult to detect. The distal centriole exhibits then a small excrescence of granular form but somewhat elongated in the direction of the mother-centriole (fig. A).



FIG. A.—Spermatid in the first period. Zeiss apochr. imm. 2 mm., oe. 12. Fixation and stain: Benda. The chondriosomes have purposely not been drawn.

In order to make the description more precise, I might add that under the spermatids going through the process noted above one finds two discontinuous layers; one of spermatogonia, the other of first spermatocytes. The latter exhibit all stages of the first phase of the growth period and the beginning of the second. Above is a layer of spermatids in all stages from the end of the second period (fig. 12) to the elimination of the spermatozoa.

#### SECOND PERIOD.

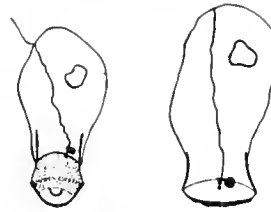
Here, as in *Phalangista*, the modifications of the nucleus would allow a subdivision of this period. First, there is a gradual condensation of the chromatin, until all structure disappears; at the same time the nucleus flattens out and assumes the shape of an egg whose long axis is perpendicular to the axial filament (figs. c, 10, and 33). Then other complicated changes occur which finally bring about a

<sup>1</sup>This filament is seen beating actively in the living cell.



peculiar form of the nucleus, roughly comparable to that of a swallow's tail. The process has been described by von Korff (pages 254-255), and is further illustrated to a certain extent by my figures, so that it is not necessary to dwell upon it.

During the first phase of the nuclear modifications the rest of the idiozome is still found as a single, solid body in the protoplasmic lobe (figs. B, c, and 10). Afterwards it disintegrates into particles formed of granules. These fragments, in preparations which show the chondriosomes, are usually more or less covered by the latter bodies and are therefore difficult to detect. One of them, however, is generally conspicuous on account of its location. It is found near the opening of the caudal tube, sometimes even within it, and remains there throughout this period (figs. 11 and 12). The headcap now covers the anterior half of the nucleus (fig. B), and the aerosome, already referred to as appearing at the end of the first period (fig. 9), has become quite conspicuous. With the gradual flattening out of the nucleus the headcap becomes more and more closely attached to it and can be recognized only when the nucleus has shrunk somewhat (in figure c the nucleus exhibits a slight amount of shrinkage); otherwise only a slight thickening of the anterior edge of the head is noticeable. Later, with the beginning of the second phase of the nuclear modifications, a peculiar appearance is observed. From the pointed extremity of the head emerges a process which projects as a sort of spur into the Sertoli cell (fig. 11).



FIGS. B AND C.—Two spermatids in the first phase of the second period. Zeiss apochr. imm. 2 mm., oc. 12. Fixation and stain: Benda. The chondriosomes have purposely not been represented.

In a still later stage (fig. 12) this structure has extended toward the other extremity of the head; thus the anterior edge of the nucleus appears, so to speak, duplicated. The two parts are connected at their extremities by an oblique line, and between by two rather thick trails of a sharply stainable substance. This condition is plainly visible after all fixations except that of Regaud. Comparing figures 11 and 12, one gets the impression that something has become disjointed from the nucleus but still adheres to it by means of this stainable material. The two trails persist for a long time in the protoplasm of the Sertoli cell, so that one may infer that the structure has not been put back into its original position. What becomes of it I could not ascertain. It seems probable, however, that here we have to deal with the elimination of the headcap, a process which von Korff was able to observe more clearly in *Phalangista*. The disappearance of the structure described would then be due to its digestion by the Sertoli cell.

We left the centrioles at the end of the first period as three granules: the proximal centriole, the distal centriole, and its process. While I feel justified in asserting that the further modifications of these granules are very similar to those described for other mammals, and belong to a type in many respects very close to those found in *Phalangista*, yet the process could not be followed in any great detail. The lack of good iron hematoxylin has already been mentioned. Three other factors also intervene to make the study difficult: (1) The eventual presence within the caudal tube (and in close proximity to the centrioles) of a fragment of the idiozome; (2)

the close contact of the proximal centriole with the nucleus and later the location of the centrioles between the branches of the nucleus; (3) the excrescence of the distal centriole, which grows so large that it can cover the whole centriolar apparatus. So much, however, could be seen. Very soon it appears that the centrioles are no longer in relation with the middle of the posterior side of the nucleus (von Korff's "laterale Insertion," fig. B); then the distal centriole flattens out and finally breaks in two, a distal ring and a proximal granule which carries the axial filament (fig. C). Meanwhile, the centriolar excrescence described above undergoes considerable growth and becomes a spherical granule close to the other centriolar fragments (figs. B, C, and 10). When the head begins to assume its definite shape this large granule is found always located toward its branched extremity. At the end of the second period—that is, when the ring is about to begin its migration—the same body becomes elongated and pear-shaped and its thickest part shows a differentiation in the form of a small, brilliant granule (fig. 12). At the same stage the proximal centriole and the anterior fragment of the distal centriole are more widely separated and connected by a thin filament. This arrangement is very similar to that found at the same period in *Phalangista* and represented by von Korff in figures 17a, 18a, and 19 of his paper, except for the presence of the large granule. Something similar to this latter body is found in other mammals, for instance in the guinea-pig. Meves describes in that species an excrescence of the anterior fragment of the distal centriole which grows to a considerable size. Later it breaks off, but is connected with one of the fragments of the proximal centriole by a thin filament. Whether any such connection with any other part of the centriolar complex exists in the opossum I have been unable to ascertain. A centriolar process, formed by an anterior fragment of the distal centriole, but smaller than in the opossum and appearing only after the migration of the ring, is described by Oliver.

The axial filament is at first uniformly thin (fig. 11 and earlier). Toward the end of this period a differentiation takes place, inasmuch as on the posterior part a deposit of a somewhat lighter substance appears (fig. 12). Thus, the future main piece can be distinguished from the middle piece. As in *Phalangista*, it is clear from that time on that the protoplasm extends farther back than the middle piece, a condition which differs from what is observed in other mammals, such as the guinea-pig, rat, etc.

The second period is characterized by the sudden appearance and disappearance of the caudal tube. While very little can be said in this case about its differentiation and regression, it should be stated that no indication was found of its nuclear origin, a view which has found some supporters but which, in the presence of Meves's observations and more recently those of Oliver, can be safely discarded. Nor was there any indication of its participation in the constitution of the middle piece, as Oliver supposes. In fact, the peculiar shape of the head and the great width of the caudal tube make such a participation hardly imaginable.

During this period of spermiogenesis the cells of the subjacent layer are found to be first spermatocytes in the second phase of the growth period, dividing primary

and secondary spermatocytes, and young spermatids up to the stage represented in figure 8. To be strictly accurate, I should add that below such cells as are represented in figures 10 and 11 one finds spermatocytes that have reached the end of the growth period. The subsequent modifications of the head of the spermatid take place while the spermatocytes are dividing. The next generation of spermatids below the stage corresponding to figure 12 has progressed as far as the stage represented in figure 8.

#### THIRD PERIOD.

At the end of this period the remnants of the idiozome have disappeared. The spermatid then contains a number of small granules in one or several heaps (figures 15*a* and 15*b*), whose size and staining properties suggest that they may be genetically related to these remnants. They are eliminated with the residual body.

Concerning the centrioles, the migration of the ring is the characteristic feature of this period. In figure 13 the ring is shown when the migration is half completed; in figure 14 at the end of it. The process is certainly a rapid one, as both stages are found side by side. From what has been said above as to the relationship between the protoplasmic body of the spermatid and the middle piece, it follows that the ring does not migrate as far as the posterior extremity of the protoplasmic lobe (fig. 14). During this period, in preparations made after Benda's method, the ring does not stain intensely, as do the other parts of the centriolar complex, and finally can not be seen at all (fig. 15*a*). Other changes in the centrioles are the following: Instead of two granules connected by a thin filament (fig. 12), we find in the next stage (fig. 13) three granules (a fourth one, which happens to be situated just below the ring, is a mitochondrion). A similar arrangement in *Phalangista* was met with by von Korff, who thinks that the two anterior granules are fragments of the proximal centriole, of which the posterior one moves gradually towards the distal centriole and finally comes to lie quite near it. By analogy one might conclude that a similar process has taken place here, although it was not actually observed.

The large, pear-shaped granule becomes more elongated as the ring migrates, as if the influence that carries away the ring had exerted itself on the former body also. It remains visible until after the deposit of the chondriosomes on the axial filament (fig. 14), but in the ripe spermatozoon it has disappeared (fig. 25). Intermediate stages frequently show one large granule or two smaller ones, which stain like centrioles and are found in the neighborhood of the head (figs. 16 and 24). To me it seems probable that they proceed from the centriolar granule. In describing a similar element in the guinea-pig Meves states that it gradually diminishes and is finally reduced to a very small size. The centriolar process described by Oliver breaks off from the anterior part of the distal centriole; it persists in the spermatozoon and is connected with a corresponding fragment of the proximal centriole by a thin filament.

The sheath covering the axial filament in the region of the main piece increases very much in thickness during this period. On the anterior extremity of the main piece it appears as an annular swelling (figs. 13 and 14); towards the posterior end it gradually becomes thinner. Within it the axial filament can usually be followed

for a certain distance. The sheath exhibits a delicacy of structure, a transverse striation, not represented in my drawings. This has already been described by von Korff, but in my opinion is not so marked as his text-figure 4 would indicate. It can be seen clearly in certain preparations only, and occasionally Ramon y Cajal's method gives very good images of it. A similar structure has been observed also by Retzius; not in *Didelphys*, however (1909, pages 125–126), but in the other marsupials studied by him (1906). In one of these (*Bettongia*), instead of a transverse striation there is a beautifully developed spiral. The existence of these conditions is interesting, as it shows that such structures are not necessarily formed by chondriosomes. I have also in mind, in this connection, the spirals, etc., described by Retzius (1902, 1910) and by Koltzoff (1908); see also Duesberg, 1912, p. 687.

In the region of the middle piece the axial filament is very little, if any, thicker than at the end of the previous period (fig. 13). It should be recalled in this connection that in the guinea-pig, during the second period, Meves found a small vesicle in that region of the axial filament. The latter appears to be the direct prolongation of the walls of this vesicle; furthermore, it is thinner within the vesicle than without. For these reasons Meves thinks that a thin sheath covers the axial filament in the region of the middle piece and that this sheath is in direct continuity with the much thicker sheath that covers the axial filament in the region of the main piece. I found in the rat (1908) a similar arrangement and adopted Meves's conclusions. Von Korff, however, does not seem to be thoroughly convinced of the existence of this sheath on the middle piece, in *Phalangista*. He states (p. 252):

“Meves nimmt ausserdem noch eine dritte Hülle an, die dem Axenfaden direkt aufsitzt; das Bläschen des Axenfadens, auf Grund dessen er ihre Existenz vermuthet, habe ich nur einmal gesehen.”

In the opossum I have never been able to find this vesicle; furthermore, the structure of the tail, as it appears in figure 13, makes it rather improbable that the sheath of the main piece extends onto the middle piece.

At the end of this period the major part of the protoplasm is eliminated. Here again there is a close resemblance to the same process in *Phalangista*. While in the guinea-pig (Meves, 1899) and in the rat (Duesberg, 1908) the protoplasm accumulates on the side of the middle piece and is there eliminated by progressive indentation, in the opossum (as well as in *Phalangista*) it flows to the anterior part of the spermatozoon (figs. 15a, 19, 20, and 21). The cast-off masses (Regaud's corps résiduels) constitute for a time a continuous layer between the expelled spermatozoa and the next generation of spermatids (fig. 16). These masses contain, besides the small granulations mentioned above, which are presumably remnants of the idiozome, a number of larger bodies and vacuoles which will be described in connection with the chondriosomes. The residual bodies are later taken up by the Sertoli cells and undergo degeneration, the termination of which is very often a transformation into fat.

#### FOURTH PERIOD.

Besides the changes that affect the chondriosomes, described in the next chapter, two important modifications take place during this period: the rotation of the

head and the so-called "copulation" of the spermatozoa. The first phenomenon is described by von Korff (p. 254) as follows:

"Der Schwanz der Spermie liegt vor der Kopulation zweier Spermienköpfe quer zu den beiden Schenkeln, nach derselben dagegen längs zu ihnen und zwar mit dem vorderen conischen Ende des Verbindungsstückes und dem Halse zwischen ihnen."

The copulation of two spermatozoa was first described by Selenka. Von Korff observed it in *Didelphys* but not in *Phalangista*. However, as Selenka states that most of the spermatozoa found in the vagina of *Phalangista* are twin cells, von Korff suggests that the copulation may take place only after the passage of the spermatozoa in the epididymis; *i. e.*, in the vas deferens. Retzius (1906) does not mention the presence of twin spermatozoa in *Phalangista*, but found them in the epididymis of *Didelphys* (1909). Jordan apparently overlooked them at first (p. 54), but corrected his opinion afterward (note 4, p. 76). I myself also found the copulating spermatozoa in the epididymis of the opossum. Preparations fixed with osmic acid and stained with Benda's method show that a distinct line of demarcation between the two heads is visible only in the posterior part (fig. 25). The fusion is, however, not as intimate as this appearance would lead one to believe; indeed the connection must be a rather loose one, as in smears the spermatozoa are often found separated. As to the significance of the copulation nothing is known. Some clue might be expected from a study of the fertilization in *Didelphys*; unfortunately, in Hartmann's paper on the subject (1916) the fact is not even mentioned.

A description of the complicated head of the spermatozoon has been given by von Korff (p. 254) and by Retzius (1909). One detail of structure has apparently been overlooked by von Korff, but is described by Retzius. This author mentions the presence, in close proximity to the insertion of the tail, of—

"einer kleinen, bei der Osmium-Rosanilin-behandlung rot, nach der Sublimat-Hämatotoxylinalaun schwarz färbaren Kugel, welche offenbar einem Centralkörper entspricht. Nach der Ablösung des Schwanzes nebst dieser Kugel bleibt gewöhnlich ein heller Fleck an der Scheibe zurück, welcher wohl als ein Grübchen aufzufassen ist, in welchem die genannte Kugel angeheftet gewesen ist."

No such structure could be found after Benda's stain (cf. figures on plate 1, and fig. 25), but it was brought into evidence after osmic fixation by iron hematoxylin (fig. D), and by acid fuchsin-methyl green after fixation with Regaud's fluid (figs. 18, 19, 20, 22, 23, and 24). As the latter series shows, the structure appears during the development of the spermatozoon, and is always to be found on the thick branch of the nucleus; *i. e.*, the one through which the twin spermatozoa become united (figs. 18, 19, 20, 22, 23, 24, and D). I never saw any evidence of the presence of a granule in this depression, as accepted by Retzius, and while I would leave this point undecided one thing is certain: If such a granule exists it has nothing to do with the centrioles.

The centriolar ring, or "Schlusscheibe," is described by Meves (1899, p. 360, footnote) and pictured by von Korff (text-figures 3 and 4) and by Retzius (1909,



FIG. D.—The heads of two copulating spermatozoa, from a section of the epididymis, stained with iron-hematoxylin after Benda's fixation. Zeiss imm. 2 mm., oc. 12.

figs. 7, 15, and 17) as a very conspicuous body. As already stated, it can not be seen in preparations after Benda (figs. 16 and 25), but appears very clearly in smears stained with iron hematoxylin. The centriolar granules located in the collar are represented by von Korff (text-figures 3 and 4) as three granules disposed very much like the same granules in my figure 13. Retzius, however, describes, instead of the two posterior granules:

“einen kleinen dunklen Halbring welcher wohl auch zum Centralkörperapparat gehört und dann als die vordere Abteilung des distalen Körpers zu betrachten ist.”

My observations agree with von Korff's, with this difference; that the two posterior granules, which are still apparent in spermatozoa taken from the testicle (fig. 16), appear usually more or less completely fused together in smears from the epididymis (fig. 25). Slight differences in the shape and length of the head and middle piece, noted between smears and sections, are probably due to a sort of capillary action exerted in the former (cf. figs. 16 and 25).

### CHONDRIOSOMES.

Since Jordan's surprising conclusions, a reinvestigation of the chondriosomes in the testicle of the opossum has always appeared to me as necessary. An effort made several years ago to collect material from the zoölogical gardens in Europe proved unsuccessful, so that the present opportunity was gladly taken.

The details of Jordan's description will be discussed when the necessity arises, in connection with my own observations, and I shall limit myself to a résumé of his main conclusions. While he admits that chondriosomes are present in the spermatids, and that part at least of these bodies form the spiral filament of the spermatozoon, he denies their existence at certain stages of the process of spermatogenesis. Concerning their absence in the spermatogonia and in the Sertoli cells, he does not express himself very definitely. He then continues:

“But my preparations leave no doubt respecting the absence of mitochondria during the early growth period of the primary spermatocytes. For this generation of cells, they first appear during the later growth period—and during a period coincident with a transitory achromatic reticular phase of the nucleus. This observation is the more significant in view of the fact that both within and without the nuclear wall are similar darker-staining bodies. Subsequently such bodies (now deeply staining, sharply contoured spheres and dumb-bells) are aligned on the nuclear membrane externally. All the evidence here hints to a nuclear origin of mitochondria, *i. e.*, they appear to be transformed chromidia (p. 59.)”

Of this nuclear origin Jordan is not, however, altogether sure. The main point upon which he insists is their discontinuity.

“I believe that the fact of their apparent absence in the young spermatocytes of the opossum (and possibly other forms) is one of the strongest arguments against the Benda-Meves-Duesberg theory of their continuity and hereditary significance” (p. 69).

There is no doubt that Jordan's conclusions, if verified, are of considerable importance. I have from the beginning been aware of it, and have been anxiously awaiting an opportunity to study the same material. At the same time I could not

help expressing (1912) great skepticism, which the results of my present investigations entirely justify. The main conclusion reached, in fact, is that there is no discontinuity in the chondriosomes of the seminal cells in the opossum. Chondriosomes are present at all stages of spermatogenesis from the spermatogonia and the Sertoli cells to the ripe spermatozoa. This conclusion, arrived at through the study of the adult testicle, is corroborated by the study of the organ in young animals. I come now to the details of my observations.

#### SERTOLI CELLS.

Chondriosomes are exceedingly numerous in the Sertoli cells (fig. 1) and exhibit a marked resistance to destructive influences, including the action of acetic acid. One can obtain preparations in which most of the chondriosomes, especially in the early stages of spermatogenesis, are destroyed, while they are preserved in the Sertoli cells. In such preparations the cell-body, with its processes, is sharply brought into evidence. The nucleus is very darkly staining and, as in other mammals, shows indentations. Droplets of fat, blackened by osmic acid, are very numerous at certain stages, namely, immediately after the resorption of the residual bodies. The chondriosomes are either granules or filaments. Some of these latter are very long and may extend into the processes, but are usually confined to the basal part of the cell, around the nucleus.

#### SPERMATOGONIA.

The resting spermatogonium (fig. 2) flattened against the basal membrane, contains, besides a relatively large nucleus, an idiozome and, notwithstanding Jordan's assertion to the contrary, numerous chondriosomes, but no fat. The chondriosomes are all mitochondria, massed wherever they find space, mostly at the poles of the nucleus. The idiozome is usually hidden by them. During mitosis the cell rounds out and the mitochondria are found at the metaphase (fig. 3) scattered all around the spindle, and later between the daughter-nuclei.

#### SPERMATOCYTES.

During the first phase of the growth period the chondriosomes keep their granular form. They are gathered mostly around the idiozome at one pole of the nucleus (fig. 4). Jordan denies the existence of such a distribution, because, having overlooked the chondriosomes in this stage he endeavored to find the same condition when it no longer existed. As a matter of fact, this distribution of the chondriosomes is of common occurrence in the first phase of the growth period in mammals. Later on the location of the chondriosomes changes and they are found all around the nucleus (fig. 5). Their shape likewise is modified; most of them are now short, rather thin filaments, retaining this form well into the period of spermiogenesis. They never look like the dumb-bells represented by Jordan in figure 24, nor like the granules in his figures 25, 26, 27, 29, 30, 31, 32, 33, 34, 36, 37, and 38. Nor are they located in the cell as his chromidia shown in the same figures. As to the metachromatic granules, "which are tentatively interpreted as the earlier



forerunners of the mitochondria," it should be noted that they are already deprived of their main interest, since it has been established that chondriosomes are present in all preceding stages. A special effort, however, was made to ascertain what they could be. It appears probable that the nuclear condition to which Jordan refers is the appearance one constantly finds at the periphery of pieces fixed with reagents containing osmic acid; in fact, the typical image of a nucleus after strong osmication, as shown in figure 5. That the masses of chromatin there represented are expelled into the protoplasm is an assumption in favor of which no evidence could be found.

There is little to say regarding the behavior of the chondriosomes during the mitoses of maturation. The process is similar to what has been described in other mammals, and identical in both divisions, so that it appeared useless to give more illustrations than figure 6, which represents the metaphase of the first division. The chondriosomes are found all over the cell. Later they occupy the space between the daughter-nuclei and are segregated in equal quantities, or approximately so, between the daughter-cells.

#### SPERMIOGENESIS.

##### FIRST PERIOD.

The chondriosomes of the young spermatid have still a filamentous form (figs. 7 and 8). Immediately after the stage represented in figure 8 in Benda's preparations, and still a little later after Regaud's fixation (see figure 17, which represents a somewhat more advanced stage than figure 8), one finds only small vesicles, elongated at first (fig. 9), later perfectly spherical. These vesicles have been observed by Jordan. As it is well known that chondriosomes when poorly preserved have a tendency to swell, the interpretation suggested itself that this appearance was due to defective fixation, notwithstanding that during the interval between the end of the first period and the beginning of the second no other form of chondriosomes was found in fixed material. The study of living cells confirms this view; such stages as are represented by figure 9 are readily recognizable in teased preparations of seminiferous tubules, and no vesicles (only solid granules) can be seen in them. It is a fact, however, that during this particular period the chondriosomes are especially sensitive to the action of fixing reagents. At the same time they acquire a considerable power of resistance to the dissolving action of acetic acid. From this time on they can be found in nearly any material. I have seen them in pieces fixed with Flemming's, Bouin's, or Hermann's fluids, all of which hold 5 per cent acetic acid. They do not, however, appear quite so clearly as in Benda's or Regaud's preparations and, furthermore, they retain a swollen appearance even in these later stages, during which, in Benda's or Regaud's material, they reappear as solid granules or rods. This increase in the resistance on the part of the chondriosomes to acetic acid in the last stages of spermiogenesis is nothing new and seems to be of general occurrence. Only lately (1918) I described another instance of it in the testicle of *Fundulus*. It is worth while, however, to emphasize it in this



case, as Jordan has endeavored to meet the reproach that his technique may have been defective by saying:

"Even a poor technique that, however, reveals them (the chondriosomes) clearly and typically at a certain stage and subsequently, should reveal them at every stage (when present) represented by cells in the same tissue (page 69)."

It is, in fact, not surprising if Jordan, in his material, could find chondriosomes in the spermatids and not in earlier stages.

#### SECOND PERIOD.

At first the chondriosomes have still a vesicular appearance. Very soon, however, at least in the most peripheric parts of pieces fixed in Benda's or Meves's fluid, they assume the shape of rather large, solid granules (fig. 10). Even this appearance is somewhat artificial and due to a certain amount of swelling, as the granules in the living cell are distinctly smaller. The territory of the caudal tube is entirely free of chondriosomes. Since, in the opossum, the caudal tube fills the whole width of the spermatid, all chondriosomes are separated from the nucleus by the entire length of the tube. In the protoplasmic lobe the chondriosomes do not show any special arrangement; no stage was found during this period as in the guinea-pig (Duesberg, 1910, fig. 46), nor during the preceding period as in the rat (Regaud, 1908), and again as in the guinea-pig (Duesberg, 1910, figs. 57 and 58) in which all the chondriosomes are collected at the periphery of the cell.

In a somewhat later stage, when the head begins to assume its definite shape (fig. 11), the chondriosomes are constantly found, both in Regaud's and in Benda's material, in groups of thin, bacillus-shaped rods. Still later (fig. 12) they appear as granules, forming several heaps in which the mitochondria are crowded very closely together. The same stage is marked by the first appearance of the so-called "von Ebner's tingierbare Körner" in the form of two or three granules or droplets, which usually are so close together that they fuse, assuming thus a shape different from that of the chondriosomes (fig. 12, upper left corner). These bodies can be easily distinguished from the chondriosomes, notwithstanding Jordan's opinion to the contrary. Even after Benda's method, which stains them purple, or iron hematoxylin which stains them black, their shade is different from that of the chondriosomes. Their form is also different. These bodies are fixed by all reagents except Regaud's. They can be brought into evidence in a specific way, even in material in which the chondriosomes are well preserved, by staining the sections with a nuclear stain—for example, with safranin. An interesting and convincing experiment consists in unstaining a preparation made with Benda's method after having drawn a given cell and having carefully noted its location by means of the mechanical stage; then staining the preparation with safranin and redrawing the same cell. While the chondriosomes do not take up the safranin, the "tingierbare Körner" do so with great avidity.

#### THIRD PERIOD.

At the time the ring begins to migrate the chondriosomes are still granules, but are scattered all over the cytoplasm (fig. 13). It has already been pointed out

that the migration of the ring is very rapid. The next change, *i. e.*, the collection of the chondriosomes on the axial filament, also takes place with remarkable rapidity, for stages like those represented in figures 13 and 14 are found side by side, while intermediate stages are very scarce. It would seem that after the ring has traveled a short distance on the axial filament a sort of attraction is exerted on the chondriosomes. This impression is rather strengthened by a study of preparations fixed with Regaud's fluid, in which rods are found instead of granules (fig. 18).

As neither the "tingierbare Körner" nor the small granules which I have regarded as remnants of the idiozome are fixed by Regaud's fluid, these preparations are especially convenient for the study of the chondriosomes in the last stages of spermiogenesis. In figure 18 (and also in figure 14) the middle piece appears covered with chondriosomes, while a number of these bodies are still scattered in the cytoplasm. These latter ones will never find a place on the axial filament. Figure 19 shows how they are carried away by the protoplasm flowing toward the head to form the residual body; they are finally accumulated below the nucleus (figs. 20 and 21). At the same time they dispose themselves in a peculiar and quite characteristic manner close to the periphery of the protoplasm (figs. 15*a*, 20, and 21), a disposition which is still more evident on cross-section (fig. 15*b*). Finally they are eliminated. I therefore agree on this point with Jordan, but nevertheless doubt very much that his conclusion was supported by his own observations. Had he seen the process just described he certainly would have mentioned it, but neither in his drawings nor in his text does he give any indication of it. In my opinion, what Jordan probably saw was the elimination of the "tingierbare Körner" and other granules especially well preserved in fixing reagents containing osmic acid.

All the protoplasmic granules and detritus are very conspicuous in preparations after Benda's method. The "tingierbare Körner" (figs. 13, 14, 15*a*, and 15*b*) are somewhat larger than in the preceding stage (*cf.* fig. 12). Next to these are the small granules interpreted as remnants of the idiozome (figs. 15*a* and 15*b*). In the residual body cast off by the spermatozoon, there appear numerous vacuoles and large granules (fig. 16), some stained in purple, others in brown. The granules, or part of them at least, are probably formed by the eliminated chondriosomes which degenerate. Romeis (1912) has described a "Verklumpung" of the chondriosomes in degenerating spermatozoa found in the so-called "poche séminale" of *Ascaris*; the masses formed by the chondriosomes retain for a time their original stain, but later, in Benda's preparations, take up the sulfalizarin.<sup>1</sup> Regaud's material shows in the residual body more vacuoles than Benda's (fig. 21), the former reagent apparently dissolving some of the granules preserved in the latter.

In connection with the elimination of chondriosomes at the end of the spermiogenetic process in mammals, I would recall that Regaud (1908) described its occurrence in the spermatid of the rat, while I would not admit it, any more than in the

<sup>1</sup>A paper by Bang and Sjövall (1916), which I would have liked to consult in this connection, was not available.

guinea-pig (1910). The present experience, however, has led me to reconsider this opinion, and I contemplate reinvestigating both the rat and the guinea-pig at an early date.

The number of the chondriosomes eliminated varies, but is usually very small (figures 15*a*, 15*b*, 19, 20, and 21). On the other hand, the number of chondriosomes on the middle piece is fairly constant, as will be shown later. The quantity eliminated is consequently a function of the quantity held by the spermatid, all the chondriosomes (no matter how numerous) that do not find a place on the middle piece being eliminated. To the significance of this elimination Jordan seems to attach much importance:

"The loss of a considerable amount of mitochondrial substance in the cast-off portion of the spermatid \* \* \* during the process of metamorphosis, militates against the interpretation of mitochondrial continuity and a hereditary rôle of this substance (page 70)."<sup>1</sup>

Setting aside the fact that the amount eliminated is not considerable, I fail entirely, as stated before (1912, page 624), to see what theoretical importance it can have.

To return to the chondriosomes surrounding the axial filament, let us note their further evolution in Regaud's preparations. While the superfluous chondriosomes are migrating toward the anterior part of the spermatid to be later eliminated, the others become gradually distributed with considerable regularity (figs. 19, 20, 21; also fig. 15*a*). When the elimination is completed (fig. 22) all the little rods are arranged obliquely in relation to the axial filament and at the same time are laid out in regular longitudinal rows suggesting a fir-apple or an ear of corn. The same regularity appears also in preparations after Benda, although, as already mentioned, instead of rods we find in these rather large granules (fig. 15*a*). The number of elements in each longitudinal row can be estimated up to seven or eight, and in cross-section, seven (fig. 15*b*); the total number of chondriosomes would consequently be 49 to 56. At the anterior part of the middle piece preparations after Regaud show invariably some irregularity in the disposition of the chondriosomes, inasmuch as some of them (usually two) are disposed almost perpendicular to the others (figs. 21 and 22). These, I assume, are going to form the first winding of the spiral.

#### FOURTH PERIOD.

From the description given above it results that, at the time of the elimination of the residual body, no spiral has been formed. Its formation belongs entirely to the fourth period; in other words, it takes place in the spermatozoon eliminated in the lumen of the seminiferous tubule. First, the rods dispose themselves perpendicular to the axial filament and appear somewhat thinner; the two anterior ones seem to have fused together into one curved filament (fig. 23). Then, by confluence of the rods the spiral is formed. In material fixed in Benda's fluid it is perfect in its regularity (fig. 16); after Regaud's fixation it is somewhat irregular (fig. 24).

<sup>1</sup> A similar opinion was again expressed by Jordan in 1914 (p. 167).

As clearly shown in figures 15a and 16, there is a great discrepancy between the size of the chondriosomes and the thickness of the spiral after Benda's fixation. I do not doubt that the appearance represented in figure 15a is somewhat artificial and due to a certain amount of swelling. Certainly the chondriosomes after Regaud's fixation are more similar in form to those in the living cell than they are after Benda's fixation.

The formation of the spiral filament at the expense of the chondriosomes, in the spermatozoon of marsupials, was first described by Benda (1897) for *Phalangista*. Von Korff came to the same conclusion both for *Phalangista* and *Didelphys*; it must be stated, however, that his representation of the spiral filament in the last-named species (text-figure 3) is somewhat schematic. In a later paper Benda (1906) confirms his former description for several marsupials. Jordan also has observed the spiral in the opossum. Finally, in the present paper I have followed its formation, step by step. In fact, it is such a conspicuous constituent of the spermatozoon that it is hard to understand how Retzius failed to see it. That author has published two papers on the spermatozoon of marsupials. In 1906 he studied *Bettongia cuniculus*, *Macropus billiardieri*, *Petrogale penicillata*, *Onychogale lunata*, and *Phalangista vulpina*. The structure of the middle piece is in all these species the same; it is covered by a relatively small number of rather large granules, disposed in longitudinal rows. For *Phalangista* Retzius expressly states that the regular disposition of the granules simulates a cross-striation, but he fails to compare his results with the entirely different ones of Benda and von Korff, although he mentions them. The next paper (1909) deals exclusively with *Didelphys*. Here again the middle piece is found covered with granules:

"Diese Körner liegen in geraden Reihen, mit zehn Körnern in jeder Längsreihe; von der Seite betrachtet zeigt das Verbindungsstück drei solche Längsreihen. Nach dieser Berechnung dürfte die Anzahl der Körner sich auf etwa 40 belaufen. . . . Sie liegen auch am reifen Spermium nicht in spiralförmiger Ordnung, sondern regelmässig der Quere nach, und sie verwandeln sich jedenfalls nicht zu einer Spiralfaser. (p. 125)."

I disagree with this description, first, in the estimate of the number of rows; second, as to the form of the chondriosomal sheath of the ripe spermatozoon, where, like Benda, von Korff, and Jordan, I found a spiral filament. This difference might be explained in two ways: First, the species studied by Retzius may not have been *Didelphys virginiana*; this hypothesis, however, is rather improbable and would not account for the discrepancy between his conclusions and those of von Korff and Benda concerning *Phalangista*. The second hypothesis, which would explain all differences, is that the material studied by Retzius was poorly preserved and showed a chondriosomal sheath that had fallen to pieces.

While writing on the structure of the mammalian spermatozoon I wish to correct certain errors appearing in the second edition of Bonnet's Embryology (1912, p. 28), in the reproduction of some of my drawings showing the development of the spermatozoon of the guinea-pig (1910). What Bonnet calls "*vordere Halsknötchen*" in figures *d* and *c*, is really the posterior edge of the headcap; while his "*hintere Halsknötchen*" are the fragments of the proximal centriole and should be

designated as "*vordere Halsknötchen*." In figure *g* this last term is correctly used, but in the same figure, as well as in figure *f*, the chondriosomal sheath of the middle piece is referred to as "*Spiral-faden und -hülle*;" in the guinea-pig, however, the chondriosomes do not form a spiral filament. I must add that I am not by any means responsible for these mistakes.

#### DISCUSSION.

As to the ultimate fate of the chondriosomes in spermatogenesis, the present investigation leads to the conclusion, readily foreseen, that the chondriosomes build a part of the spermatozoon—in this case a spiral filament surrounding the middle piece.

As to their origin, Jordan's assumption of their discontinuity and their nuclear nature in the seminal cells of the opossum can, in my opinion at least, after this reinvestigation be considered as a failure. To me this conclusion is no more surprising than the first one. It must be stated, however, that in later years the theory of the nuclear origin of the chondriosomes has again been taken up by Alexeieff, Walton, and K. E. Schreiner.

As far as I know, Alexeieff has published a number of notes on the subject, all dealing with protozoa (1916). He finds in these organisms bodies which he calls "mitochondries," and which he believes to be of nuclear origin; hence he proposes calling them *chromidia*. I must say that nowhere can I find any argument in favor of this author's conclusions.

Walton (1916) thinks he has demonstrated that the chondriosomes of the seminal cells in *Ascaris canis* Werner are formed at the expense of nuclear material in the spermatocytes, and he draws therefrom far-reaching conclusions. As chondriosomes do, however, exist in the spermatogonia of *Ascaris* (Duesberg 1912, p. 638, and Fauré-Frémiet, 1913), Walton's premises are incorrect, and any further discussion is unnecessary. The explanation of his failure is very simple: he fixed with strong Flemming's and Carnoy's fluids, neither of which can be trusted for the preservation of the chondriosomes.

More serious appears Schreiner's attempt. So far he has, to my knowledge, published observations only on the fat-cells of the subcutaneous tissue of *Myxine*. He promises to deal in subsequent papers with pigment-cells, blood-cells, and cells of the connective tissue, with glandular and seminal cells, the study of which brings him to the same conclusion as the study of the fat-cells. In these he finds a number of rods, "*Plasmastäbchen*," stainable with acid fuchsin (after Altmann's or Altmann-Kull's method), or with iron-hematoxylin, "*welche zur Bildung der Fettvakuolen Anlass geben*." Schreiner's view on the formation of fat is a confirmation of those already expressed by a number of authors, among them Metzner, Dubreuil, and Hoven. He differs from them, however, inasmuch as, according to him, the "*Plasmastäbchen*" are formed from nuclear substance, not from chromatin, as in Goldschmidt's chromidial theory, but from nucleolar substance. He concludes that his observations formally contradict what he calls "*die Meves-Duesbergsehe Lehre*."

Before undertaking the critique of Schreiner's paper, a few remarks of a general character would seem not to be amiss. Schreiner's assertion of the nuclear origin of his "Plasmafäden" recalls to my mind two other papers in which a similar assertion was made: one by Wassilieff (1907) on the seminal cells of *Blatta germanica*, the other by Jordan on *Didelphys*. In both cases it was claimed that the chondriosomes were formed in the spermatocytes only and in both cases I found (a positive result against a negative one) that the chondriosomes were present in the spermatogonia and were transmitted during mitosis to the spermatocytes. For *Blatta* the formation of chondriosomes at the expense of nuclear material was supposed to appear so clearly that Goldschmidt speaks of "die schönen Befunde von Wassilieff, deren unbedingte Beweiskraft für den, der die Präparate kennt, die noch viel klarer sind als die Zeichnungen, keinem Zweifel unterliegen kann (1909, p. 110)." When, however, I studied the same material (1910), I could find no evidence to substantiate the nuclear origin of the chondriosomes and, so far as I am aware, no answer has ever been made to my criticism of Wassilieff's conclusions. In fact, the sharpest critics of Goldschmidt's theory have since been found in his own laboratory, a point to which I shall return later.

These two experiences, together with the increased knowledge I have acquired of cytoplasmic structures, have made me very skeptical of such claims as Schreiner's. As to the present case, I might add that (like others, who have not forgotten the story of the enumeration of chromosomes in *Zoogonus mirus*) I am not inclined to accept Schreiner's assertions as "ready money." When one attempts, however, a specific and detailed consideration of his present observations, it readily appears that a thorough discussion is hardly possible, as Schreiner's communication is only a preliminary one, in which he repeatedly refers to his future paper and on points of no minor importance. Let us take for example the question of the seriation of the different aspects of the "Plasmafäden." *A priori*, there is no reason why we should accept that figure 20 represents a stage of fragmentation of these bodies, while certain granular filaments in figures 4, 5, 6, and 7 represent their formation. A correct seriation is, in fact, very difficult, for in most cells not only smooth filaments, but also granular ones, which Schreiner supposes to be here in process of formation, there of fragmentation, are present (see pages 159 and 164). The author himself admits the difficulty; one of his criteria is the condition of the nuclei:

"Auch der Kern hat in den Zellen, wo die Segmentierung stattfindet, ein von demjenigen ganz verschiedenes Aussehen, das wir von den Zellen kennen, innerhalb deren Cytoplasma die Stäbchen gebildet werden. Betreffs dieses letzteren Punktes muss auf meine ausführliche Arbeit verwiesen werden (p. 163)."

As I have not seen Schreiner's completed paper, I can not express any opinion as to the value of his arguments and will therefore limit myself to pointing out the difficulty. I might state incidentally that there is a contradiction (at least what appears to be a contradiction in the face of available data) between figure 22 (which shows smooth filaments only and a nearly spherical nucleus) and the description on page 160, where we read that "diejenigen Fettzellen, die in ihrem Cytoplasma zahlreiche grosse Kügelchen enthalten, sphärische Kerne mit ebenfalls runden

Nukleolen aufweisen, während diejenigen Zellen, deren Plasmakügelchen sich zu Stäbchen zu entwickeln angefangen haben, in der Regel gelappte Kerne besitzen." As to the crucial point of the origin of the "Plasmafäden," I would suggest as a possible cause of error the fact that Schreiner has made use of only such methods as stain nucleoli and chondriosomes alike—*i. e.*, iron-hematoxylin and acid fuchsin—and not of Benda's method, which stains them differently. It should also be recalled that other authors who have studied the fat-cells have declared themselves for the cytoplasmic nature of their chondriosomes; for instance Dubreuil, whose completed paper (1913) Schreiner has overlooked.

Taking for granted, however, that Schreiner's description is accurate, nothing proves yet that his interpretation is correct, for the process described as an expulsion of substance from the nucleus might just as well be the reverse. And even if we accept Schreiner's interpretation, we find that the question of the origin of the chondriosomes has really not been touched, since these bodies are present, according to Schreiner's own description, in all cells before the process he describes takes place.

These are the points which the preliminary account of Schreiner suggest to me. I wish to add that the bibliographic review contains a number of omissions and errors. Among the first I note, aside from the one already mentioned (Dubreuil's paper), Schreiner's denomination of the theory of the cytoplasmic nature of the chondriosomes as the "Meves-Duesbergsche Lehre." As a matter of fact, practically all cytologists agree on this point, one of the last to so express himself being Maximow (1916). As to errors, we read, for instance, on page 148 that "die Anhänger der Chromidialtheorie stimmen mit denen der Plastosomentheorie darin vollkommen überein, dass die Chromidien und Plastosomen die nämlichen Gebilde sind"—an opinion never expressed by me (see Duesberg, 1912). Further, we find that the criticism of Retzius (1914) is very highly praised. I have already pointed out why Retzius can not be considered as an authority in this matter (Duesberg, 1915, pages 62–63) and would refer the reader also to Meves' answer (1914, 2) to Retzius. On page 169, Schreiner writes:

"Da ich das Material, bei dem Goldschmidt die Beobachtungen machte, welche die Grundlage seiner Chromidialtheorie bilden (die Gewebszellen der Ascariden) nicht aus eigener Untersuchung kenne, wage ich zu den verschiedenen Meinungen, die über die Natur seiner Chromidialstränge von verschiedenen Seiten (Vejdovski, Bilek, Duesberg) geäußert sind, keine Stellung zu nehmen. Doch muss ich gestehen, dass es mir schwer fällt zu glauben, dass sich Goldschmidt von seinen Präparaten dermassen hat tauschen lassen, wie die genannten Autoren behaupten."

To this, I would answer that I have expressed no definite opinion on Goldschmidt's "Chromidialstränge," as I recognize "dass es kaum möglich ist, sich ohne eigene Kenntnis des Objektes kategorisch auszusprechen (1912, p. 907)." I insisted, however, that all authors agree on one point, *i. e.*, that Goldschmidt was mistaken; and I would emphasize here that these authors are not only the ones mentioned by Schreiner, but also Sjövall and Lundegårdh (who declare themselves unconvinced by Goldschmidt's description), Hirschler, Ehrlich, Jörgensen, and

von Kemnitz (the last three working in Goldschmidt's laboratory) who, like Vejdovsky and Bilek, speak from personal experience. In all fairness it should be added that Jörgensen and Ehrlich agree with Goldschmidt as to the nuclear origin of some of the "Chromidialstränge," but in a way which can hardly be satisfactory to him: "In den von Goldschmidt beschriebenen Fällen handelt es sich um durch das Messer herausgerissene Nukleolen oder Chromatinbröcken des Kernes selbst." I shall not dwell further upon the chromidial theory, for the whole case was thoroughly exposed by me in 1912 and, as I like to recall, my criticism has never been answered.

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NOTE.—Through the courtesy of Prof. E. B. Wilson, I had the opportunity, after the above was written, to read a recent paper by Schreiner: "Zur Kenntnis der Zellgranula. Untersuchungen über den feineren Bau der Haut von *Myxine glutinosa*. Erster Teil, erste Hälfte. Arch. f. mikr. Anat. Abt. I. Vol. 89." I can not in the present paper, discuss at length Schreiner's article, but I would state that in my opinion all I have said before concerning (1) the accuracy of Schreiner's description, (2) the correctness of his interpretation, and (3) the legitimacy of his conclusions, still holds. As to the second point, it should in all fairness be stated that Schreiner himself is aware of the difficulty and discusses it at some length. On page 140 I find the following argument in favor of his view:

"Schon der Umstand dass die feinen Verbindungsfäden zwischen den kleinen f. en Plasmakörnchen und dem Nukleolus innerhalb des Kernes oft eine recht beträchtliche Länge haben können, und dass die Nukleolarsubstanz sich in diese Fäden nicht selten allmählich fortsetzt, scheint zugunsten der ersteren Erklärungsweise zu sprechen. Eine in dieser Hinsicht noch grössere Bedeutung wird man aber der Tatsache beimessen müssen, dass in einigen Zellen vom Nukleolus ähnliche Fäden ausgehen, die an der inneren Wand der Kernmembran endigen, ohne mit irgend einem Plasmakörnchen in Verbindung zu treten. Solche Bilder wird man schwer auf andere Weise deuten können, als dass wir hier eine Vorbereitung für die Ausstossung der Nukleolarsubstanz durch die Kernmembran vor uns haben."

How the conditions described should rather speak in favor of Schreiner's opinion than against it, I fail entirely to see.



## THE APPARATUS OF GOLGI.

The entire literature concerning the apparatus of Golgi, up to the spring of 1914, was reviewed by me at the meeting of the Anatomische Gesellschaft in Innsbruck. Recent contributions to the subject have been made by Addison (1916), Basile (1914), Birek (1914), de Castro (1916), Cowdry (1916), Deineka (1914), Hortega (1914), Monti (1915), Pensa (1915), Pappenheimer (1916),<sup>1</sup> Ramon y Cajal (1914), Sanchez (1916), and Speciale (1914).<sup>2</sup> The most interesting points in these contributions will be reported at the end of this chapter. For the previous literature I take the liberty of referring the reader to my review and will confine myself at this time to recalling the observations of Sjövall (1906), Perroneito (1910), and Weigl (1912), which are closely connected with my subject.

By means of a special method Sjövall brought into evidence a number of rods at the periphery of the idiozome, in the spermatocytes of the mouse. In the spermatids the same method stains that part of the idiozome which is not used in the formation of the headcap and which is finally eliminated.

Perroneito was the first to apply the silver impregnation (Golgi's method with acidum arseniosum) to the testicle. In the spermatogonia of the rabbit he found, at one pole of the nucleus, a typical reticular apparatus. In the spermatocytes the apparatus is somewhat smaller and sometimes irregular, inasmuch as from the reticulum a long process may be sent out into the cell. As to its behavior during mitosis, Perroneito does not express himself definitely, although he thinks that there are some indications of a process similar to that which he finds in the spermatocytes of *Paludina* (dyctiocinesis). In the spermatids the reticulum is even smaller than in the spermatocytes. What becomes of it he could not ascertain, but is inclined to believe that one part of it is eliminated, while the rest remains in the spermatozoon.

Weigl (1912) studied the ripe spermatozoon in the guinea-pig by means of Golgi's, Ramon y Cajal's, and Kopsch's methods. In the protoplasmic sheath of the collar he found some rods and granules, which he is inclined to consider as an apparatus of Golgi. He wonders whether the apparatus is carried by the spermatozoon into the egg and has, perhaps, something to do with the formation of the apparatus in the embryonic cells.

In the spermatogonia of the opossum I find very often two kinds of bodies impregnated by Ramon y Cajal's method (fig. 26). There are first a number of granules which resemble very much the mitochondria of these cells. There is, further, a much more voluminous body flattened against one pole of the nucleus. The central part of this body is formed by a light substance and its periphery is

<sup>1</sup> While Pappenheimer felt the necessity for "collating the widely scattered and rather inaccessible literature," he merely reproduces the data collected by me up to 1914 and gives only an incomplete account of the recent papers.

<sup>2</sup> Voivov (1916) should probably be added to this list. He describes, in the spermatocytes of *Gryllotalpa*, a body which is stainable by the chondriosomal methods and which can also be impregnated by the silver methods. Single in the young spermatocytes, it later separates into four parts which come to rest against the nucleus. During the mitoses of maturation these bodies are equally segregated between the daughter-cells, so that each spermatid gets one of them. This body is eliminated at the end of the spermiogenesis.

There is undoubtedly a striking resemblance between this body on one side, and Platner's "Nebenkerstäbchen" and Perroneito's "dyctiosomes" on the other.

marked by a sharp, black line. Although no such reticulum as Perroncito figures for other mammals could be brought into evidence, I do not doubt that this darkly impregnated substance corresponds to his apparatus of Golgi. That the method was applied with full success is demonstrated by the presence of a beautifully developed reticulum in the interstitial cells (fig. 34). Further, the Sertoli cells in my preparations show a structure similar to that represented in Perroncito's figure 83. This pictures a Sertoli cell of the guinea-pig, containing a system of anastomosed rods, which Perroncito considers a typical reticular apparatus. I can confirm this observation for the guinea-pig; in the opossum a similar condition is met with, the difference being that the filaments are somewhat thicker and often appear hollow.

In the spermatocytes studied during the first phase of their evolution (fig. 27) the apparatus has increased considerably in size and shows certain details in structure which may have been present in the spermatogonia, but which could not, perhaps, be seen on account of the small dimensions of the body. Sections perpendicular to the apparatus show that the dark envelope is missing in the middle part of the side toward the nucleus (fig. 27, left). Tangential sections (fig. 27, right) reveal the fact that this dark envelope is not a solid shell, but is formed of a number of filaments of varying thickness. Some of these filaments are anastomosed, yet the impression given is never that of a typical reticulum, as Perroncito figures in other species. Sometimes the apparatus is formed of two lobes reunited by a filament.

During the second part of the growth period the change in the shape of the apparatus apparently is in relation to the increased size of the spermatocyte; having more space, the apparatus rounds out (fig. 28). During the prophase of the first division, and as late as the metaphase (fig. 29), the apparatus appears as a sort of unrolled coil located anywhere in the cytoplasm. In the anaphase (fig. 30) a number of granules, or clumps of granules, may be found scattered between the daughter-nuclei, the thread having apparently fallen to pieces. The reconstitution of the apparatus in the daughter-cells takes place gradually; there is a stage during which two apparatuses are found in the second spermatocytes (fig. 31). Jordan, who figures and describes a division of the "sphere" in what he considers as the first prophase, must have mistaken the second generation for the first, as a fragmentation of the idiozome does not take place, as demonstrated in my description, before the first metaphase.

If we compare the behavior of the apparatus during mitosis in the testicle of mammals with its behavior in invertebrates, as first described from silver preparations by Perroncito for *Paludina*, and much earlier by Platner for *Helix*—for literature see Duesberg (1914), pages 34–35—we find that in mammals the process lacks the regularity exhibited in the lower forms. The apparatus behaves, as far as I can judge from the accounts given, like the idioectosome of Stockard and Papanicolaou.

In the young spermatid the apparatus looks more like a reticulum than at any other stage. Then a differentiation takes place; a vacuole appears which increases

in size until it finally comes in contact with the nuclear membrane. In the meantime the reticular structure is gradually collected on one side of the vacuole (fig. 32), and finally detaches itself from the latter, to pass into the protoplasmic lobe. There it appears as a granular body up to the stage represented in figure 33, which corresponds approximately to figure 10, drawn from Benda's preparations. A similar arrangement, although in a somewhat younger stage, is represented by Perroncito for the cat in his figure 89. Later, the apparatus breaks into several smaller bodies of the same structure. Finally, only small granules are found which are eliminated with the protoplasm. Thus it appears that the apparatus does not take any part in the constitution of the ripe spermatozoon, in contradistinction to Perroncito's and Weigl's suggestion reported above, and that similar structures found in the embryonic cells either are derived from the egg's apparatus or are formed *de novo*.

If we compare the silver preparations with those made from material fixed with Flemming's, Hermann's, or Benda's fluid, we find that in the resting cells (cf. figs. 5 and 28) the images are similar. The apparatus is nothing but the outer, darker-staining shell of the idiozome—Stockard and Papanicolaou's *idioectosome*. The points that stand out most clearly in silver preparations are: (1) the behavior of the apparatus during mitosis; (2) that it is identical with the so-called "Idiozomrest."

Finally, I wish to point out the complete similarity between my conclusions and those of Sjövall, arrived at by entirely different methods; and to add that a study of the testicle of the guinea-pig by means of Ramon y Cajal's method has given me identical results.

#### DISCUSSION OF APPARATUS OF GOLGI.

In my review on the apparatus of Golgi (1914) I endeavored to clear up the question of relationship between this element and other constituents of the protoplasm. It would perhaps be of interest to reexamine these conclusions in the light of my present and other recent observations.

A point that I especially desired to settle, as far as possible, was that of the relationship between the apparatus and Holmgren's trophospongium. I came to the conclusion (pages 60-61) that two categories of cells should be distinguished: The neurones and the non-nervous cells with a localized trophospongium on the one side, and the non-nervous cells with a diffuse trophospongium on the other. As to the latter, the identity of both formations can be rejected without further discussion; for, while the trophospongium extends all over the cytoplasm, the apparatus of Golgi is localized at one pole of the nucleus.<sup>1</sup> As to the former, both formations appeared to me to be identical. The difficulty, however, is to reconcile Holmgren's opinion (according to which the trophospongium is in communication with the outside) with that of a large number of authors who hold that the apparatus of Golgi is limited to the cell. I expressed the view that Holmgren must have confused the apparatus with the exogenous processes which are known to pene-

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<sup>1</sup> Exception made for the lutein cells.

trate certain nerve cells (since then another example of this penetration has been reported by Ross, 1915), an opinion which is strongly supported by the publications of Nusbaum's pupils.

At the time I was making my report at Innsbruck, Holmgren (1914) published a paper in which he reaffirmed the continuity of the "Trophozyten" and their processes with the intracellular apparatus of the ganglion cell, his conclusions being based especially upon the study of preparations made after Kopsch's method. In another paper (1915) he insists again upon the correctness of his views and expresses at the same time his dissatisfaction with the opinion given in my review, an opinion which I have just summarized. Regarding Holmgren's paper I wish to say this: When I undertook my review I was entirely unprejudiced. My conclusions were based, first, upon a thorough study of the literature (in fact Holmgren can not reproach me with any gaps or misrepresentation of his views—quite an achievement considering his prolixity and versatility); second, upon a study of preparations of my own; third, upon a number of Holmgren's preparations. Details concerning the latter can not be given, as the preparations themselves have been returned to Holmgren and the notes I took at Liège are not available. I recall very clearly, however, that, notwithstanding the persuasive notes which Holmgren sent with the preparations, I failed to be convinced. My skeptical attitude towards Holmgren's theory, therefore, is well based, and he himself is in part responsible for it. I can not help wondering why, if he really had the facts, he did not come to Innsbruck and show his preparations instead of writing articles, for I had already informed him of my conclusions.

Since my review and Holmgren's first article (1914), a paper by Ramon y Cajal (1914) has appeared, in which the author discusses the same question. His opinion on most of the points is entirely in accord with my own. He calls the intracellular apparatus *aparato tubular de Golgi-Holmgren*, meaning two things: First, that the two formations are identical, a view in agreement with my own, exception being made, however, for the non-nervous cells with diffuse trophospongium, for which it can not hold; second, that these formations are a system of ducts, an opinion I must regard with some skepticism. Speaking of the possible connections of the apparatus with processes of trophocytes, Ramon y Cajal expresses himself as follows (p. 211):

"Lo que importa notar particularmente es que, si positivamente en ciertos elementos ganglionares de los vertebrados, existen conductos radiados para alojar apendices de los trofocitos, estos apendices no se hallan en continuacion substancial con el aparato de Golgi. Ni acabamos de persuadirnos de que las cistadas celulas nutritivas representen disposicion general. A nuestro juicio, no es posible descubrir el menor resto de ellas en los epithelios, ni en la inmensa mayoria de las neuronas centrales, ni en los elementos del embrion di uno a dos dias, donde el aparato de Golgi esta bien diferenciado."

Another point of great interest is the question of relationship between the apparatus and the chondriosomes. In 1914 I thought it safe enough to conclude that in nerve cells (p. 18), as well as in other cells (pp. 35-36), the apparatus is a structure different from the chondriosomes, although the possibility of genetic

connections was to be taken into consideration. Since that time three interesting papers have appeared which deal with this question. Two of them, one by Deineka (1914), the other by Rina Monti (1915), favor the identity of the chondriosomes and the apparatus, while Ramon y Cajal (1914) takes the opposite stand.

Deineka's conclusions are based upon his study of the connective tissue during the process of ossification. The method used was that of Golgi, with acidum arseniosum, but the duration of the fixation was reduced to about 30 minutes, instead of 6 to 7 hours. In the osteogenous tissue this method impregnates, besides a number of granules and filaments which are obviously chondriosomes, some thicker filaments localized at one pole of the nucleus. These correspond to the apparatus, but for Deineka they are of the same nature as chondriosomes, of which they represent only one part. In the osteoblasts very numerous short filaments and granules are accumulated on one side of the nucleus, leaving free amid them a clear space which is obviously the "sphere." In the bone-cells the image changes with the age of the cell. In none of these cases could Deineka make out a difference between an apparatus and the chondriosomes.

Ramon y Cajal, who opposes Deineka's conclusions, believes (p. 158) the latter's method does not impregnate the apparatus. In osteoblasts he shows it (fig. 23) precisely around that clear spot ("sphere") described by Deineka. To me it seems possible that the thick filaments localized at one pole of the nucleus, which Deineka represents in the cells of the osteogenous tissue, and perhaps in some of the bone-cells, correspond to the apparatus; while the other elements in the cells of the connective tissue are undoubtedly chondriosomes. The fact, however, that all these bodies are or can be impregnated at the same time, does not prove that they are the same. Deineka himself (1912) has shown that under certain conditions of fixation the apparatus alone is impregnated, while his present method apparently preserved the chondriosomes better than the apparatus.

While Deineka's standpoint can hardly be defended, Monti's opinion appears to me worthy of consideration. This author has studied the nerve-cells of invertebrates and vertebrates by means of the chondriosomal and silver methods. In her opinion the chondriosomes of the adult neurone and Golgi's apparatus are one and the same thing. The differences between the two sets of preparations are due to the following causes: First, that the latter is a method of impregnation, while the former consists of a progressive differentiation. Second, that in the first case thin sections, in the second thick ones, are used. Monti, however, is far from admitting the identity of Golgi's apparatus with the chondriosomes in all cases. In young nerve-cells she finds two reticula; a large one extending all over the cell-body and a small one localized at one pole of the nucleus. The former is the same as one finds in the adult cell and is formed by the chondriosomes; it is similar to the reticulum described first by Pensa, in cartilage cells, which is also formed by chondriosomes;<sup>1</sup> the other is identical with the small reticulum described by von Bergen in the

<sup>1</sup>That the apparatus first described by Pensa (1901) corresponds to the chondriosomes can not be doubted; but I do not believe that the chondriosomes of these cells form a reticulum (see Duesberg, 1914, p. 23), even if it may appear so after the silver impregnation.

cartilage cell and other similar formations and has the same behavior during mitosis. It does not exist in the adult cell in which the centrosphere has disappeared and the reproductive activity has ceased.

If Monti were correct, all the difficulties concerning the existence of a relationship between the so-called apparatus of the adult nerve-cell and the cellular centers (see below) would be settled at once, as the structure that exhibits such a relationship—the small reticulum—would exist only in the young cell. At the same time a revision of the nomenclature would appear necessary. Which formation should we persist in calling Golgi's apparatus—the small reticulum or the chondriosomes? There is no doubt that the latter, or any similar term would be preserved by the majority of authors, and we would find ourselves in the rather awkward position of no longer being able to speak of Golgi's apparatus in the adult nerve-cell, where it was first described. To be entirely logical, the term *Golgi's apparatus* should be given up altogether, as otherwise it would perpetuate an error, and some other denomination be substituted for the small reticulum whose main characteristic in the resting cell is its topographical relation with the cellular centers. I must state, however, that although Monti's opinion appears to me interesting, I am not entirely convinced that the morphological differences between chondriosomes and reticular apparatus can be satisfactorily explained as she proposes. I have in mind a number of figures published by different authors which can hardly be reconciled with her opinion, including some of the figures given by Cowdry (1912) and Ramon y Cajal (1914).

One of the points emphasized in my review is the close topographical relationship between the apparatus and the cellular centers, a relationship to which Ballowitz (1900) was the first to call attention, and whose importance can not be overestimated, as it is likely to constitute an excellent criterion. For details of the bibliography I refer the reader to my review, and especially to pages 37–39. I would call attention, however, to certain conclusions embodied therein.

The non-nervous cells can be divided into three groups: (1) A group in which the relationship between the apparatus and centers is established; (2) a group in which the same relationship appears extremely probable; (3) a group in which the relationship appears possible. Concerning the nerve-cells, I pointed out that this relationship is clear enough in the embryonic stages; not so, however, in the adult cell. For these one could admit that the apparatus gradually outgrows the centrotheca and surrounds the nucleus, as we know it does in certain cells. But even in the adult its complicated form does not exclude, as I suggested, such a relationship, for we know of cases in which the centrotheca undergoes somewhat similar changes. This last hypothesis does not, however, seem to be supported by recent investigations.

Hortega (1916) has recently published a number of data concerning the centrioles of the adult nerve-cell; the comparison of his results for the Purkinje cells, for instance, with those obtained by Sanchez (1916) for the reticular apparatus of the same cells is certainly not in favor of any close relationship between apparatus and centrioles. Monti's opinion, as indicated above, settles the difficulty, but it can not be accepted without further investigation. Ramon y Cajal (1914), for

whom the chondriosomes and apparatus are two different things, believes that "en las neuronas adultas, la despolarizaci6n del aparato de Golgi y su difusi6n perinuclear coincide probablemente con la atrofia y desaparici6n de la esfera y centrosoma (p. 209)." This opinion is plausible only if, as Cajal himself indicates, it is limited to mammals, and further, if it is well understood that "esfera" and "centrosoma" do not mean "centrioles," a confusion which is too often made.

As to the non-nervous cells Ramon y Cajal (1914, p. 208), after extensive observations on the apparatus in embryonic, cartilaginous, epidermic, and glandular cells, in odontoblasts and osteoblasts, in fat and goblet cells, subscribes wholeheartedly to my opinion:

"Desde las clasicas investigaciones de Ballowitz, efectuadas en el epitelio posterior de la cornea, y confirmadas despues para otros tejidos por numerosos autores (Negri, Pensa, Barinetti, Terni, Perocito, Deineka, etc.) quedo perfectamente establecido que, en toda celula portadora de un reticulo endocelular polarizado y concentrado, existe en el centro de este un hueco donde se aloja la esfera atractiva. Numerosos indicios confirmatorios de esta conexi6n hemos consignado tambien nosotros al describir la disposici6n del aparato de Golgi en las grandulas, osteoblastos y osteoclastos y durante las fases ontogenicas de las neuronas y corp6sculos epitelicos. En este punto suscribimos de buena gana el pensamiento de Duesberg (1914), para quien el aparato de Golgi estaria primeramente ligado al sistema de la esfera atractiva. . . ."

A further confirmation of the same opinion is found in Basile's researches (1914) on the modifications of the apparatus in the renal epithelium after nephrectomy. While normally the apparatus is located between nucleus and lumen, after the operation it has moved to the basal part of the cell, and with it the centrioles.

The relationship between the apparatus and the centers of the resting, non-nervous cell appears, therefore, more and more safely established.<sup>1</sup> The structure of a young resting cell could, in my opinion, be adequately represented by the accompanying schema. It should be well understood, however, that the apparatus is not always a reticulum. Another remark of importance is that, as a rule, in epithelial tissues the apparatus is located in that part of the cell which Cajal (1914) has called the "polo mundial o de relaci6n exterior" (see Duesberg, 1914, p. 21.)<sup>2</sup> As to the chondriosomes, their form and location vary.

During mitosis the apparatus falls to pieces and its fragments are scattered all over the cell-body, or else the primitively isolated parts of the apparatus behave in the same way. There appears to be in some cases considerable regularity in the shape of these fragments, their number and their distribution between the daughter cells. The behavior of the chondriosomes during mitosis is again variable, as I emphasized recently (1917, p. 478 et seq.; see also Meves, 1914, 1).

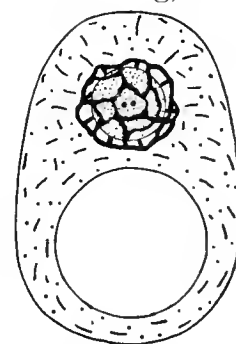


FIG. E.

<sup>1</sup>Cowdry (1916) does not agree with me in my "attempt to define the apparatus in terms of its relation to the centrosome, because our knowledge of the centrosome itself is so deplorably inadequate (page 40)." If this applies to the non-nervous cells as well as to the nerve-cells, I certainly do not agree with Cowdry's skeptical attitude, either towards the relationship of the apparatus to the centers, or towards our knowledge of the centrosome.

<sup>2</sup>The similar location of the centrioles is, since the researches of K. W. Zimmerman and Heidenhain, well known.



In older cells the apparatus may lose its connections with the cellular centers; for instance, in certain epithelial cells, the lutein cells, cartilage cells during the process of ossification (for literature see Duesberg, 1914, pages 40–41, and Ramon y Cajal, 1914), and finally (if we do not accept Monti's interpretation) in the nerve-cells. Then, instead of being localized at one pole of the nucleus, the apparatus surrounds this body more or less completely.

The nomenclature of these cytoplasmic constituents is unfortunately exceedingly complicated. As to the chondriosomes, I have already explained why I consider the term "mitochondria," taken in a general sense, as illogical and confusing (1917, pp. 469–470). The mass of differentiated protoplasm which in many resting cells (young ovocytes, seminal cells, cells of the connective tissue, cartilage cells, etc.) incloses the centrioles, constitutes another object of confusion. How little some appreciate the difference between this mass and the original "sphere attractive" of van Beneden, is best illustrated by the following quotation from a recent paper by Shaffer (1917, p. 416):

"There is no essential difference between the 'attraction sphere' of van Beneden, the 'centrosphere' of Strasburger, and the 'astrosphere' of Fol and Boveri; and so far as I have been able to ascertain, there is no fundamental difference between these last-named structures and the 'idiozome' of Meves. One thing is clear—that these structures all refer to the achromatic substance of the spindle, situated at the poles and usually inclosing the central corpuscles."

This is, of course, entirely incorrect. The idiozome has nothing to do with the "achromatic substance of the spindle situated at the poles." The necessity of distinguishing between the mass of protoplasm which surrounds the centrioles in many resting cells (idiozome or centrotheca) on one side, and the attraction sphere of van Beneden on the other, has been emphasized long ago by von Erlanger and by Meves. (For literature see Meves, 1897 and 1914, 1). What term, then, should we use? I agree with those authors who believe that the term "sphere" should be rejected in order to avoid any confusion with the "attraction sphere" or "astrosphere" of the dividing cell. To avoid this confusion Meves has proposed the term *idiozome*, and later, in order to emphasize the relationship of the body with the centrioles, the term *centrotheca*. To the first term Regaud has objected (the same objection could be made to "centrotheca") for the reason that the relationship with the centers does not persist during mitosis nor (in seminal cells) during spermiogenesis. He proposes the term *idiosome*, which Stockard and Papanicolaou have adopted. There is no doubt that this name, although rather vague (or perhaps because of this), has much in its favor, if only investigators could agree upon it. It should be added that the term "centrosome" is also used in a very loose manner, and a great number of authors unfortunately take it as a synonym for "centrioles," Shaffer, for instance.

Still more complicated and confused is the nomenclature of that body which surrounds the idiozome. It is now well established that it corresponds to what was formerly called "Nebenkern" in the seminal cells of *Helix*, by Platner (Hermann's "Archoplasmenschleifen"), to some of Van der Stricht's "pseudochromosomes," to



Ballowitz's "Centrophormien," and to Heidenhain's "Centralkapsel." More complete bibliographical data will be found in Duesberg (1914) and also in Kuschakewitsch and Terni. In recent times the same body has been called frequently "Golgi's apparatus;" other denominations are "von Bergen's reticulum" (Monti), "formazioni periidiozomiche" (Terni), "dyctyosomes" (for the fragments during mitosis, Perroncito), "idioectosome" and "idiophthartosome" (Stockard and Papanicolaou). Kuschakewitsch proposes calling it "Sphärotheca" and "Sphärosomen" (during mitosis), while the complex formed by the "Sphärotheca" and the "idiozome" is designated as "Statosphäre."

Since, as noted above, I have rejected the term "sphere," I can not accept Kuschakewitsch's nomenclature. To these terms, which emphasize the relationship between the discussed body and the centers or the idiosome (Centralkapseln, Centrophormien, formazioni periidiozomiche, idioectosome), the same objection may be raised as to the terms "idiozome" and "centrotheca," for such a relationship is not durable. Other denominations, such as "Nebenkern," "von Bergen's reticulum," "idiophthartosome," etc., are obviously bad. Personally, I would prefer the term "Golgi's apparatus," or rather "Golgi's intracellular apparatus" (Golgischer Binnenapparat, as proposed by Nusbaum), which is already very widely used and does not prejudice any special morphological appearance. The only difficulty is the one emphasized above, when discussing Monti's conclusions.

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## EXPLANATION OF FIGURES.

All figures were outlined with a Zeiss camera lucida, at the level of the stage of the microscope. Zeiss apochromatic immersion 2 mm., ocular 12. Artificial light (gas).

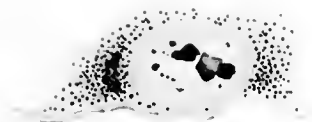
### PLATE I.

Fixation, Benda; stain, Benda.

- FIG. 1. A Sertoli-cell. The three big granules in the basal part, near the nucleus, are fat-droplets.  
 FIG. 2. Resting spermatogonium.  
 FIG. 3. Dividing spermatogonium, metaphase.  
 FIG. 4. First spermatocyte, pachyten stadium  
 FIG. 5. First spermatocyte in the second phase of the growth-period.  
 FIG. 6. Dividing first spermatocyte, metaphase. The small granules to the right of the spindle and below the equator are fat-droplets.  
 FIGS. 7, 8, 9. First period of spermiogenesis.  
 FIG. 7. Young spermatid. The granules in the upper left corner are fat-droplets.  
 FIG. 8. More advanced stage.  
 FIG. 9. More advanced stage. Note the small acrosome on anterior part of the nucleus.  
 FIGS. 10, 11, 12. Second period of spermiogenesis.  
 FIG. 10. The head has assumed the form of an egg-shaped body, whose long axis is perpendicular to the axial filament.  
 FIG. 11. The head begins to assume its definite form. First indication of disjoining of headcap. Note an idiozomic remnant near the opening of the caudal tube, on the left.  
 FIG. 12. Further stage of the disjoining of the headcap. Idiozomic remnant near the opening of the caudal tube. In the upper left corner, first appearance of "tingierbare Körner." Note the sheath developing around the axial filament in the future main piece.  
 FIGS. 13, 14, 15, *a* and *b*. Third period of spermiogenesis.  
 FIG. 13. Migration of the ring. The granule right below the ring is a mitochondrion, not a centriole. In the protoplasmic lobe, remnant of the idiozome (to the right of the ring), mitochondria and "tingierbare Körner," darkly stained and fused together.  
 FIG. 14. Deposit of mitochondria on the axial filament in the middle piece. "Tingierbare Körner" to the right of the main piece.  
 FIG. 15*a*. Elimination of protoplasm with some mitochondria, idiozomic remnants (?) and "tingierbare Körner" covering part of the head and the anterior part of the middle piece.  
 FIG. 15*b*. Transverse section through the region of the middle piece showing the peripheric disposition of the chondriosomes which are going to be eliminated. To the right, cross-section of the middle-piece; to the left and somewhat below, "tingierbare Körner;" in the lower right corner, idiozomic remnants (?).  
 FIG. 16. Fourth period. The spiral filament is formed. Between the spermatozoa, expelled into the lumen, and the seminal epithelium, a layer of residual bodies with big granules and vacuoles.

### PLATE 2.

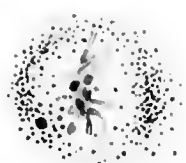
- FIGS. 17-24. Fixation, Regaud; stain, acid fuchsin-methyl green.  
 FIG. 17. Spermatid in a stage somewhat more advanced than the cell represented in figure 8. Note the difference in size after the action of two different reagents.  
 FIGS. 18-21. Third period of spermiogenesis. Successive stages of the deposit of chondriosomes on the middle piece and the elimination of the residual body, with some chondriosomes.  
 FIGS. 22-24. Fourth period of spermiogenesis. Successive stages of the formation of the spirale.  
 FIG. 25. From a smear of sperm from the epididymis. Fixation vapors of osmic acid. Stain, Benda. Copulating spermatozoa.  
 FIGS. 26-34. From preparations after Cajal and handled further as described in the text. Counterstain: methyl-green for figures 26-30, 33 and 34; Ehrlich's hematoxylin for figures 31 and 32.  
 FIG. 26. Spermatogonium with impregnation of mitochondria (?) and of the intracellular apparatus.  
 FIG. 27. Two first spermatocytes in the first phase of the growth-period. To the left a perpendicular section, to the right a tangential section.  
 FIG. 28. First spermatocyte, second phase of the growth-period.  
 FIG. 29. First division, metaphase.  
 FIG. 30. First division, anaphase.  
 FIG. 31. Second spermatocyte, with two apparatuses.  
 FIG. 32. Spermatid in a stage of development corresponding approximately to figures 8 and 17.  
 FIG. 33. Further stage of development of the spermatid, corresponding approximately to figure 10.  
 FIG. 34. Interstitial cell.



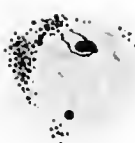
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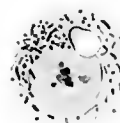
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15b



15a



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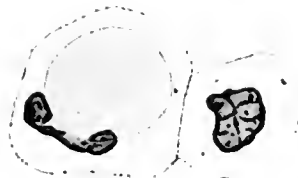
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CONTRIBUTIONS TO EMBRYOLOGY, No. 29.

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ON THE WIDESPREAD OCCURRENCE OF RETICULAR FIBRILS  
PRODUCED BY CAPILLARY ENDOTHELIUM.

BY GEORGE W. CORNER,  
*Assistant Professor of Anatomy in the University of California.*

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With two plates.



# ON THE WIDESPREAD OCCURRENCE OF RETICULAR FIBRILS PRODUCED BY CAPILLARY ENDOTHELIUM.

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By GEORGE W. CORNER.

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In adding his contribution to those here gathered, the writer deems it most appropriate to present a study which not only took origin during the course of an investigation suggested by Dr. Mall, but which led back into another field that he had made particularly his own, and in which his interest and advice would have been most eagerly sought, had a happier providence allowed.

In one of his best-known and most important papers Dr. Mall, in 1891, announced his discovery that the framework of many organs and tissues of the mammalian body is composed neither of white fibrous nor of yellow elastic connective tissue, but of a third type of supporting substance composed of fine interlacing fibrils which not only differ from the white fibers in appearance, but are more resistant to both acid and alkaline solvents and are not so readily attacked by digestive ferments. He applied the name "reticulum" to the new tissue because the fibrils of the lymph-nodes, already bearing this name, were the first which he found to present the characteristics just mentioned. The supporting fibrils of the spleen, gastric and intestinal mucosa, liver, lung, thyroid, heart-muscle, the basement membranes of the testis, and the entire supporting structure of the kidney, including the basement-membranes, were all demonstrated in this first paper to be of the same type. That the internal connective tissue of many other organs falls in the same category was later shown in publications by various of Mall's pupils, of which the most interesting in the present connection are those upon the corpus luteum by J. G. Clark (1898), and the adrenal gland by J. M. Flint (1900). In 1902 Mall published his important account of the development of the connective tissues, showing that in the intestine, and presumably in other organs, the reticular fibrils are developed within the cytoplasm of the mesenchymal syncytium.

To this last statement, however, he noted one striking exception. In the liver the reticulum arises from von Kupffer's endothelial cells:

"The observations upon the development of the reticulum of the liver are entirely out of harmony with those of the development of connective tissue elsewhere. In all other places the syncytium arises from the mesenchyme, but here it is from the endothelial lining of blood vessels. . . . The fibrils are in no way connected with the liver cells and true mesenchyme cells are not present at all."

This discovery was confirmed by J. Kon in 1908. A denial by Madame Schumkow-Trubin (1909) would seem to be erroneous; the present writer's preparations agree with the description of Mall and Kon.

After the work of several investigators had proved the identity of the fibrils shown in the liver by the digestion method with the "Gitterfasern" long since

observed by Henle and Kupffer, for which Oppel had in 1890 discovered a method of selective impregnation by a silver-chromate method, further proof of Mall's conclusions as to the difference between reticulum and white connective tissue was afforded by the application of Bielschowsky's silver-nitrate method to the staining of these tissues. As shown by Ferguson (1912), successful impregnation of adult tissues gives a totally distinct coloration of the two kinds of fibers.

#### CORPUS LUTEUM.

The present writer's interest in this subject began during his study of the origin of the corpus luteum (Corner, 1919). It will be remembered that Sobotta, in his well-known account of the formation of the corpus luteum of the mouse, states that the connective-tissue framework is formed by the migration of the theca interna cells inward among the granulosa and their simultaneous conversion into "Spindelzellen." In the sow's ovary the author did not observe such a change of the theca cells, but found that they remain interspersed among the granulosa lutein cells of the fully formed corpus luteum, and like the latter are merely enveloped by the interlacing fibrils of the connective tissue. Nor is there sufficient participation of the theca externa to provide fibroblasts to lay down the supporting framework; indeed, in the fully developed corpus luteum one does not see, except perhaps in the septa close about large vessels, any spindle-shaped cells which can be definitely stated not to be components of capillary walls. As before mentioned, Clark, using Mall's methods, has shown the connective tissue within the corpus luteum to possess the characteristics of reticulum, and this observation is readily confirmed by the Bielschowsky stain. In the presence of such a dilemma—an organ plentifully supplied with reticular connective tissue, yet without connective-tissue cells—one could not fail to recall Mall's account of the condition in the liver. The endothelium is the only possible source of the reticulum when we exclude the granulosa and theca lutein cells.

In order to test this hypothesis it was necessary to demonstrate the reticulum by methods which do not at the same time destroy the other elements of the organ, but which on the contrary give the sharpest possible pictures of the fibrils in their relation to surrounding tissues. To this end the Bielschowsky-Maresch silver-nitrate method proved best adapted. It was used exactly as directed by Ferguson (1912), after fixation in 10 per cent formol, alcoholic formol, Bouin's fluid, or Zenker's fluid. Sections were cut in paraffine at 4 microns. After impregnation they were usually counterstained with alcoholic carmine. The Bielschowsky-Maresch method has been extremely capricious in the author's hands, but when successful the jet-black fibrils show against the red counterstain in the clearest possible manner. Occasionally the cytoplasm is colored a deep golden brown by the silver, and counterstaining is unnecessary. In order to display the capillary net, vascular injections were made, but these for various reasons proved chemically incompatible with the reagents used in impregnating the sections. Hence it was necessary to rely upon the presence of erythrocytes in the capillaries, in cases where the exact relations of the endothelium would otherwise have been dubious. This

was secured, in the preparation of some of the specimens illustrated in this paper, either by selecting engorged tissues, by ligating the vena cava under anesthesia, or (in the case of the fetal kidney) by injecting oxalated blood of the same species into the arterial system under considerable pressure.

The test of our hypothesis is shown in figures 1<sup>1</sup> and 2, which display the condition found in the fully developed corpus luteum of the cow and sow respectively. There can be no doubt that in this organ the endothelial cytoplasm itself contains the fibrils of reticulum which support the tissues by embracing each lutein cell in their basket-like meshes. The delicate complexity of the network, well seen in figure 2, is explained by the richness of the capillary bed, which touches every cell in the whole gland. As in the figures, the fibrils rarely if ever embrace the endothelial nuclei, but usually pass between them and the lutein cells, leaving the nuclei to bulge into the capillary lumen. Fibrils are never seen in the endothelium of vessels whose walls are more than one cell-layer thick, but such arterioles and venules are provided with a perivascular reticulum.

#### ADRENAL, HYPOPHYSIS, THYROID.

The adrenal gland is another organ whose framework is known to consist of reticular fibrils (Flint, 1900), yet contains no fibroblasts. Professor Evans's studies of vital staining with benzidine dyes provide a delicate test for the presence of fixed as well as wandering connective-tissue cells, and they show that there are no fibroblasts in the adrenal cortex (personal communication). Figure 3 illustrates the condition shown by the Bielschowsky method in the zona reticularis of the rat's adrenal; figure 4, in the zona fasciculata. Here again there can be no doubt that it is the capillary endothelium that subserves the function of reticulum formation.

In the anterior lobe of the hypophysis the relation between the circulating blood and the epithelial cells is so close that not only is there no space for fibroblasts, but some have even doubted the continuity of the capillary tubes, suggesting incomplete walls, as in the liver. In this gland the fibroblastic activity of the endothelium is very readily demonstrated, for it is seen in the walls of relatively large sinusoids (fig. 5).

In text-book descriptions of the thyroid gland it is stated or implied that the reticular framework, mentioned by Mall (1891) and described by Flint (1903), is laid down by connective tissue in interfollicular strands which carry the blood-vessels; yet careful study of thin sections of the thyroid readily confirms the statement of Flint that "interfollicular connective tissue is scant save in the neighborhood of the great vessels." Major (1909) has pointed out the exceedingly close contact between the perifollicular capillaries and the follicle-cells. In preparations of the rat's thyroid, made to illustrate this paper, the strands of connective tissue along the larger vessels are composed of collagenous fibers, with only here and there a cell which may be interpreted as a fibroblast containing reticular fibrils. Away from the arteries and veins, between the follicles, the only cellular elements present

<sup>1</sup> Figure 1 is from a preparation of Mr. J. F. Cobb, for whose assistance, cut short by his entrance into the military service, I am much indebted.

are the endothelial walls, and these contain the reticular fibrils. Figure 6 well represents the condition.

#### RENAL BASEMENT MEMBRANES.

Besides the glands of internal secretion, there are other organs in which fixed interstitial connective-tissue cells are known to be scarce or absent, yet which possess well-developed supporting fibers, and in which the capillaries are intimately applied to the secreting cells whose functions they subserve. These conditions exist in the cortex of the kidney, and here again the point of departure for our investigation is found in the work of Dr. Mall. In his first paper of 1891 he showed that after digestion of sections of the kidney with pancreatin the entire structure, from capsule to pelvis, including the basement membranes, is a single mass of anastomosing fibrils which possess all the characteristics of reticulum. This statement, amply confirmed by Rühle (1897), was in disagreement with the older belief in a homogeneous basement membrane. The perplexity was cleared up in 1901 by Mall's discovery that both structures exist, the structureless membrane applied closely to the bases of the epithelial cells, and itself intimately invested without by the cylinder of interlacing fibrils. In sections prepared with connective-tissue stains it is the outer coat that gives the traditional appearance of a basement membrane; in the macerated and teased preparations of older histologists probably the inner coat was most obvious. Mall's whole conception has recently been confirmed and extended by von Frisch (1915).

It is now generally held, therefore, that there is an intertubular stroma throughout the kidney, composed of interwoven fibrils, some of which are condensed against the tubules to form the *membrana propria*. They are said to be produced by flattened nucleated cells which are more common in the kidneys of young animals; according to Disse (1902), who has given a full description of the "renal stroma," in adult life the cells are found chiefly in the neighborhood of the papillæ and the fibrils become independent of the cells.

Application of the Bielschowsky method with counterstaining permits us to observe the exact relation of the fibrils to surrounding cells with a clearness not known to former observers, and it at once becomes evident that we shall have to revise the conception of the stroma of the kidney as well as of the endocrine glands previously described. In the renal cortex, between the convoluted tubules and about the glomeruli, the "stroma" is no more nor less than a network of reticular fibrils imbedded in the cytoplasm of the capillary endothelium or deposited by the latter against the tubules and Bowman's capsules. True fibroblasts are very infrequent, perhaps altogether absent. Figure 7 shows the renal cortex of the adult rat.

In the papillæ and along the medullary rays there is, on the other hand, a true stroma consisting of fibroblasts which produce reticular fibrils. Figure 9 is taken from a medullary ray in the renal cortex of a fetal pig 145 mm. long, and well illustrates the contrasting condition. The medullary fibrils are in general much thicker than those of the cortex, but no chemical difference has as yet developed, such as might be expected from the different origin of the two types.

The relations of the fibrils to the elements of the glomeruli are naturally of much interest. At favorable points where the epithelial layer of Bowman's capsule is fairly thick, or where it has become separated from the adjacent tissues, as in figure 8, it is seen that the fibrils are related to the capsule exactly as to the thicker epithelium of the convoluted tubules. These fibrils are at some points so far away from capillaries that one hesitates to make the statement that they are produced by endothelial cells here also. In some species wandering connective-tissue cells are found at the point of entrance of the arteriole into the glomerulus, and it is possible that fibroblasts are also present and form the reticulum immediately surrounding the glomeruli. The endothelium of the glomerular tuft is in no case provided with reticulum, affording a marked contrast to that of the intertubular capillaries.

At first thought it seems impossible that the capillary network, with its open meshes, could lie against the renal tubules sufficiently to cover their surface at all points with a fine network of fibrils, but two considerations remove the force of this objection. (1) Even in thin sections of kidney in which the vascular system has been completely injected with a color-mass, it is found that the capillaries touch the convoluted tubules at practically all points. The familiar figure of an injected human kidney given by Disse (1902, p. 79) illustrates the point. The renal capillary network must be so rich and the individual vessels so flattened against the tubules by the pressure of neighboring tissues as to make the meshes very small and thus practically to complete the surface of endothelium which rests upon the bases of the epithelial cells. (2) Of late we are beginning to think of the capillary endothelial cells not as parts of a rather inert, fixed conducting tube, but rather as dynamic elements, in some organs actively phagocytic, in others perhaps engaged in elaborate chemical processes, at all points ever ready to vary their pattern by putting out and withdrawing sprouts in response to the circulatory needs of the tissues.

#### GENERAL CONSIDERATIONS.

We shall need to await a more complete exploration of the capillaries of the whole body in their relation to the reticulum before attempting to discuss the general significance of the facts here fragmentarily reported. For the present we can merely say that in certain organs where true connective tissue is absent, and the blood-capillaries come into direct contact with actively secreting epithelial cells, the capillary endothelial cells themselves are able to lay down the supporting framework of the gland. Where the support of tissues is provided by fibroblasts and their products, the endothelium seems devoid of reticular fibrils. It will be important to determine next, if possible, whether there are chemical differences between endothelial and fibroblastic reticulum, and whether the endothelial cells which produce fibrils are otherwise different from those which do not possess such a function.

Already, however, our observations begin to throw light upon certain problems of endothelial physiology and pathology. Since the studies of vital staining demonstrated the phagocytic properties of the cells which line the peripheral sinuses of

lymph-nodes, the exact nature of these cells has been in doubt. Evans (1914, 1915) placed them in the category of endothelial phagocytes. Downey (1915) gives a good account of the two prevailing opinions, pointing out quite correctly that the same cells produce the reticulum; but his further assumption that by that fact they can not be endothelial, would seem to be invalid when we know that endothelium in other places has the power to lay down fibrils. In the spleen and lymph-node we may assume that the endothelial cell and that which forms reticulum are one and the same.

Our results would also seem to bear on the discussion of pathologists as to the connective tissue in endothelial neoplasms. Wooley (1903) has reported the frequent presence of "intercellular fibrils" in these tumors, which he ascribes to reversion of the proliferating endothelial cells to a more primitive mesoblastic character. We have shown that such a function is in some localities characteristic of fully differentiated endothelium.

#### DESCRIPTION OF FIGURES.

All the preparations illustrated are from sections cut at 4 microns, and stained by Ferguson's modification of the Bielsehowsky-Maresch method. All except those shown in figures 1, 7, and 8 were counterstained with alcoholic carmine. The plates were drawn by Ralph W. Sweet.

##### PLATE 1.

- FIG. 1. Corpus luteum of cow, fetuses 210 mm. long. (Preparation by Mr. J. F. Cobb, jr.)  $\times 1,100$ .  
 cap. .... a blood-capillary in longitudinal section.  
 retic. .... reticular fibrils in an endothelial cell of the capillary wall.
- FIG. 2. Corpus luteum of sow, fetuses 20 mm. long, showing reticular fibrils in the capillary endothelium.  $\times 1,160$ .  
 cap. .... a blood-capillary in cross-section, containing an erythrocyte.  
 nuc. .... nucleus of an endothelial cell.  
 retic. .... reticular fibrils which can be traced to a capillary, but are not themselves actually in a vessel-wall.
- FIG. 3. Adrenal gland of rat, showing reticular fibrils in the endothelial cells of a sinusoidal blood-vessel in the zona reticularis near the point of exit of central vein from the medulla.  $\times 1,100$ .  
 sin. .... sinusoid.  
 fibr. .... fibrils connected with the endothelium but not actually in the vascular wall.
- FIG. 4. Adrenal gland of rat, showing reticular fibrils in the endothelium of a capillary in the zona fasciculata. Some of the erythrocytes are impregnated lightly, others are intensely blackened.  $\times 1,100$ .
- FIG. 5. Hypophysis of beef, showing reticular fibrils in the endothelial cells of a sinusoidal blood-vessel.  $\times 1,100$ .  
 sin. .... sinusoid.  
 fibr. .... interlacing reticular fibrils displayed where a portion of the vascular wall is cut tangentially

##### PLATE 2.

- FIG. 6. Thyroid gland of rat, showing reticular fibrils in the endothelium of a capillary between two alveoli.  $\times 1,100$ .  
 nuc. .... nucleus of an endothelial cell.  
 eryth. .... erythrocytes in the capillary lumen.
- FIG. 7. Kidney of adult rat. Small area of cortex, showing that the reticular fibrils, which here form basement-membranes for the convoluted tubules, are imbedded in the endothelium of the capillary blood-vessels. The figure also shows a portion of a very small venule, in which endothelium is free from fibrils.  $\times 880$ .  
 vein. .... venule of the smallest order.  
 eryth. .... erythrocytes in capillary.
- FIG. 8. Kidney of adult rat, showing relations of the fibrils to the epithelial elements of Bowman's capsule.  $\times 760$ .  
 epith. .... epithelial element of Bowman's capsule, which is slightly wrinkled, so that its cytoplasm is clearly visible and is seen to be distinct from the reticular fibrils.
- FIG. 9. Kidney of fetal pig 145 mm. long. Area of medullary ray showing portions of a collecting tubule and of a secreting tubule (probably a distal convoluted tubule near the ascending limb of Henle's loop). The field contains reticular fibrils produced both by fibroblasts and by endothelial cells.  $\times 760$ .  
 bl. vess. .... blood-vessels.  
 retic. .... reticular fibrils produced by endothelium, forming the basement-membrane of a secreting tubule.  
 fibrobl. .... fibroblasts producing reticulum and forming supporting sheath of a collecting tubule.











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CONTRIBUTIONS TO EMBRYOLOGY, No. 30.

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VARIABILITY IN THE SPINAL COLUMN AS REGARDS  
DEFECTIVE NEURAL ARCHES  
(RUDIMENTARY SPINA BIFIDA).

BY THEODORA WHEELER,  
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With eleven figures.

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# VARIABILITY IN THE SPINAL COLUMN AS REGARDS DEFECTIVE NEURAL ARCHES (RUDIMENTARY SPINA BIFIDA).

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BY THEODORA WHEELER.

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Studies of minor variations are continually being presented in biological fields. The reason for the persistent attention directed toward this line of study is not far to seek. To any observer the more or less frequent appearance of varied characteristics in form or function can not fail to suggest many perplexing questions as to the causes underlying such processes, and also as to their immediate and ultimate effects. As a consequence, some of the most valuable theories in the different branches of the natural sciences have been suggested, and are being worked out, by means of the evidence afforded by these variations. The theory of evolution, with all its ramifications, has been and is being developed along these lines. Since the beginning of the nineteenth century, especially, the attention centered about variations has been tremendous. Lamarek, Darwin, Mendel, and then Galton were really the first to get the upper hand in the study and treatment of variations, and more recently a rapidly enlarging group of workers in genetics have, by fresh methods, gleaned a most fruitful harvest in this field. The methods themselves have been most numerous and varied, and it is especially true of this subject that its history is to be found in the history of its methods. At first lax and slipshod, they have gradually become, through the influence of the men just mentioned, thorough and accurate. Probably the chief ones in present use are the statistical and the experimental methods and their combinations. So, as time goes on, innumerable minutiae and data are gathered on every side and made to serve their part in unraveling problems. A noteworthy instance of help afforded by such studies is the more fundamental idea which we now have of the biological conception of the *normal* or *type*. This very necessary standard, though even now far from finally understood, has developed in the minds of men from a very rigid concept into a far more plastic and adaptable principle, mainly through the insight gained by variation study.

Very often these studies may be applied with profit to specialized problems. An example of this is the frequent and rather interesting results obtained along morphological lines in the higher animal forms in demonstrations of the persistence in postnatal life of earlier phases of development, which, as a rule, become changed or obliterated during the course of growth. The present investigation deals with the variability throughout the spinal column in respect to incomplete union of the posterior laminae of the vertebrae. Associated with this subject is the question of its relation to the pathological condition of spina bifida. The incomplete ossification of the adult vertebral spinous processes is also an example of a

condition of delayed development which has been present throughout the spine at an earlier period.

The bony closure of the posterior vertebral arches begins in the lumbar region, proceeding upward and downward from this point. In a model of the chondrocranium and cervical vertebræ of a 42 mm. embryo (No. 886, Carnegie Collection) made by Dr. C. C. Macclin, the cartilaginous closure of the atlantal neural arches lags considerably behind that of the other cervical vertebræ, which evidence also substantiates this sequence in the cervical region. As is the case with other growth phenomena, there must be considerable variation in the time of completion of this process. According to Hennig (1880) the lumbar spinous processes are ossified by the third year. Macalister (1893) gives time of completion of the dorsal arch in the atlas as the fourth year, and Radlauer (1908) states that ossification of the sacral spinous processes, beginning with  $S_1$  in the third year, is finally completed with  $S_4$  and  $S_5$  in the seventh year.

Incomplete closure of the sacrum has been the subject of numerous studies. In 1902 W. R. Smith noted the variation associated with left-sided sacralization of the fifth lumbar in a female Australian aboriginal sacrum, and more recently Radlauer (1908), Adolphi (1911), Frets (1914), and Wetzels (1915) have contributed considerable data on the subject. Radlauer studied 500 sacra, representing various races, from the University of Zurich and from several German collections. He found a completely open sacral canal in 5 per cent of the cases. He found also that closure of the hiatus sacralis occurred more frequently, (1) over the fourth and between the fourth and fifth sacral vertebra, 45.6 per cent; (2) over the third and between the third and fourth sacral vertebra, 27.4 per cent; and (3) over the fifth sacral vertebra, 14 per cent.

Adolphi (1911), working in Dorpat with 292 skeletons (234 male and 58 female), found an open sacral canal in 3.4 per cent of the males and 1.7 per cent of the females—a total of 3.08 per cent.<sup>1</sup> In 50.3 per cent (48.7 per cent of males and 56.9 per cent of females) there were 4 sacral vertebræ closed;<sup>2</sup> in 24.3 per cent (25.2 per cent of males and 20.7 per cent of females) 3 vertebræ were closed;<sup>3</sup> while in 12 per cent of both males and females 5 vertebræ were closed.<sup>4</sup> In 12.7 per cent there was some degree of opening of the first sacral arch.<sup>5</sup> In a group of 203 specimens (161 male and 42 female), in which there were 5 and 6 vertebræ with no transitionally formed ones, he found that the hiatus sacralis reached to the third vertebra<sup>6</sup> in 26.6 per cent (28 per cent of the males and 21.4 per cent of the females); to the fourth vertebra<sup>7</sup> in 51.2 per cent (48.4 per cent of males and 61.9 per cent of females); and to the fifth vertebra<sup>8</sup> in 12.3 per cent (13 per cent of males and 9.5 per cent of females).

Frets, in material drawn from the population of Amsterdam, in which no Jewish skeletons were included, found that out of 750 specimens the sacral canal

<sup>1</sup>Adolphi's table 4.

<sup>2</sup>Adolphi's table 3, columns 2 and 5.

<sup>3</sup>Adolphi's table 3, columns 3 and 6.

<sup>4</sup>Adolphi's table 3, column 1.

<sup>5</sup>Adolphi's table 4, column 2.

<sup>6</sup>Adolphi's table 5, columns 7 and 9.

<sup>7</sup>Adolphi's table 5, columns 2 and 6.

<sup>8</sup>Adolphi's table 5, columns 1 and 5.

was completely open in 15, or 2 per cent. He further found closure of the hiatus sacralis to be more frequent over  $S_4$ . This occurred in the six-segmented sacra in 57.3 per cent of the specimens, and in the five-segmented sacra in 70 per cent. In a group of 265 sacra with five vertebræ there were 9 (3.4 per cent) with completely open canal, but none among 113 sacra in which the first coccygeal segment was fused with the sacrum, nor among 150 six-segmented sacra. Thus, in a total of 528 specimens representing these three types the open sacral canal exhibited this variation of 1.7 per cent.

Frets's findings in regard to the hiatus sacralis and open sacral canal in this group of 528 cases are shown in table 1 (adapted from table iv in his article in the *Morphologische Jahrbuch*, 1914). Here a definite relation appears to exist between the number of segments in the sacrum and the region of closure of the hiatus. In sacra formed of 5 vertebræ it was open higher than in those formed of 6. In the six-segmented sacra the hiatus sacralis closed over the fifth vertebra in 34 per cent of the cases, as opposed to 5 per cent in those with 5 segments; while in the latter it closes over  $S_3$  in 20 per cent of the specimens, as opposed to 7.3 per cent in the six-segmented sacra.

TABLE 1.—*Form of Hiatus canalis sacralis, 528 cases (adapted from Frets).*

Location of hiatus canalis sacralis.	Sacra with 5 segments, 265 cases.		Sacra with 5 segments and fused with $Co_1$ 113 cases.		Sacra with 6 segments, 150 cases.	
	No. of cases.	P. et.	No. of cases.	P. et.	No. of cases.	P. et.
$S_5$ .....	13	5	25	22	52	34
$S_4$ .....	186	70	83	73	86	57
$S_3$ .....	54	20	5	4.4	11	7.3
1 posterior arch present.....	2	0.8	.....	.....	1	0.67
2 posterior arches present.....	1	0.4	.....	.....	.....	.....
Sacral canal entirely open.....	9	3.4	.....	.....	.....	.....
Posterior arch of $S_1$ reduced in varying amount	57	21.5	22	19.4	51	34

In 22 Australian sacra Wetzel (1915) found 2 with completely open canal. He also mentions a study of 257 specimens from the "Breslau Anatomy" (which I have not been able to locate) in which this variation was found in 3.5 per cent of the cases. He noted that the hiatus sacralis closed over  $S_4$  in 8 cases and between  $S_4$  and  $S_5$  in 8 cases, and that there were numerous instances where the arches of  $S_1$  either did not unite at all or united so low as to correspond to the junction of  $S_1$  and  $S_2$ .

At this point it may be well to call attention to the fact that in such reports rarely is any information given, or even an estimate made as to how much of the material presented was originally acquired because of the presence of such variations or anomalies. The chances are that some of the material in almost any collection has been selected on this account, and therefore ratios based upon these cases are exaggerated. Very probably the rather wide range of variation of the open sacral canal (5.0 to 1.7 per cent) as presented by the three authors quoted above, Radlauer, Frets and Adolphi, is due as much to differences in *collection* as to true representative differences in populations or races.

In the present investigation 1,000 consecutive X-ray plates of the lumbar region in white adults (over 18 years of age) were studied at the X-ray department of the Johns Hopkins Hospital, as were also all the available cervical vertebræ in the Division of Physical Anthropology at the U. S. National Museum at Washington. From the latter source over 600 atlases and more than 3,000 other cervical vertebræ were examined. The kindness of Dr. Baetjer, of the Johns Hopkins X-ray department, and of Dr. Aleš Hrdlička, curator of the department of physical anthropology, National Museum, is acknowledged with pleasure.

As the osteological collection at the National Museum was studied mainly for cervical vertebræ, no thorough record of sacral conditions was attempted. In one group of 33 white skeletons, however, in which the sacrum was present with the rest of the spinal column, 2 cases of completely open sacral canal were noted. One of these was in a sacrum composed of 5 vertebræ; the other was also in a five-segmented sacrum, but in this case  $L_5$  was sacralized on the left side, and that vertebra showed also a bifid dorsal arch (fig. 5). In one six-segmented sacrum in this group the dorsal arches were united only over  $S_3$  and  $S_4$ . In a similar group of 22 sacra from various American Indian tribes the condition of an entirely open sacral canal was noted twice.

Examination of the X-ray plates showed 4 cases of completely open sacrum in white males and 4 in white females. These are given in table 2 and clinical aspects of the cases follow on page 102. Unfortunately, an accurate count was not kept of the entire number of plates in which the

TABLE 2.—*Types of defective sacrum.*

	No. of cases.	Alone.	Associated with $L_5$ defect.	Associated with $L_4$ and $L_5$ defect.
Male. . . .	4	3	1	0
Female. . .	4	2	1	1
Totals.	8	5	2	1

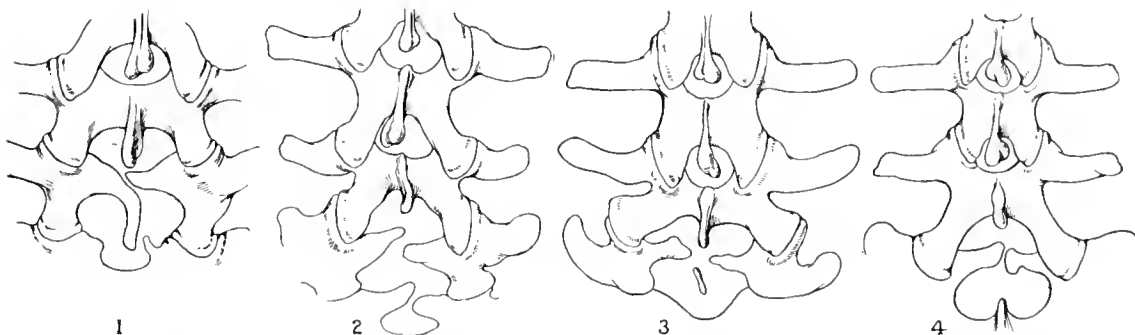
whole sacrum showed plainly, so that these cases could not be used in calculating percentages, and the groups of whites and Indians just noted are too small to be of statistical value. In the X-ray plates dorsal closure of the first sacral vertebra was found to be considerably defective in 78 cases of white males (14.57 per cent) and in 53 cases of white females (11.39 per cent). This ratio is in close accord with that of Adolphi (12 per cent), while that of Frets is much higher (24.6 per cent). The possibility of difference of opinion on this point should, however, be taken into account. One has to choose a rather arbitrary standard of what constitutes a defective first sacral arch, since the transitions from a fully formed arch to an incomplete one are so very gradual. The author found the second as well as the first sacral arch to be defective in 4 cases (3 male and 1 female). Occurring with slightly less frequency than in the sacrum, complete union of the vertebral posterior laminae is found in the last lumbar vertebra in from 2 to 3 per cent of cases. In the 1,000 consecutive X-ray plates of the lumbar region of white adults that were studied, it was found 12 times among 535 males (2.22 per cent) and 11 times among 465 females (2.38 per cent), giving a total of 2.3 per cent. Table 3 shows its occurrence and association with other defective vertebræ. The designation, *last lumbar*, has been used instead of  $L_5$  or  $L_6$ ,

as many of the X-ray plates did not show the exact number of lumbar vertebrae. The last lumbar was the only lumbar vertebra found to be so affected, except in the case of one female, where the fourth, as well as the fifth, and also the entire sacrum, showed the condition. (See case S. R.)

TABLE 3.—Occurrence of incomplete ossification of spinous process of last lumbar vertebra.

	Male.	Female.	Total.
No. of X-ray plates examined.....	535	465	1000
Cases with defective last lumbar spinous process:			
Number.....	12	11	23
Per cent.....	2.22	2.38	2.3
No. of preceding cases where last lumbar defect was associated with defect in spinous process:			
S <sub>1</sub> .....	3	2	5
Entire sacrum.....	1	2	3
Other cases where last lumbar spinous process showed irregular development	5	2	7

That the ratio obtained, representing the variation of bifid L<sub>5</sub>, is a close approximation of its occurrence in any white population of mixed nationalities would seem to be a reasonable assumption. The X-ray plates constituted a consecutive series, the patients coming to the hospital for various causes, and in general the slightest irregularity of the last lumbar vertebral process shows very clearly in the plates. There are two modifications of the above statement, however, that must be taken into consideration as unknown factors, in spite of the fact that they probably represent only a small source of error and would tend to balance one another. On the one hand it must be admitted that a hospital population would be apt to include, somewhat in excess of an ordinary population, such cases of spina bifida as present clinical manifestations. On the other hand, a certain number of L<sub>5</sub> with defective arch would not show on an X-ray plate, as the split occasionally occurs close to the median line, but shadowed from the antero-posterior view by a bulky spinous process. In any event, since only 4 of the 23 cases where L<sub>5</sub> was involved gave clinical symptoms (q. v. clinical), and as the additional group of L<sub>5</sub> cases where irregularity of the spinous process was noted numbered only 7, the ratio of occurrence would not be greatly changed by these factors. Figures 1, 2, 3, and 4, taken from the X-ray plates, show the various types of incomplete closure encountered.



FIGS. 1 to 4.—Tracings taken from X-Ray plates, showing various types of incomplete dorsal closure of the fifth lumbar vertebra. Fig. 1, J. H. H. No. 36595. Fig. 2, J. H. H. No. 45489. Fig. 3, J. H. H. No. 35876. Fig. 4, J. H. H. No. 43888.

A differentiation is to be made between the type of defective arch in which there is a medianly incomplete dorsal arch, as in these cases, and the condition studied by Manners-Smith and Hrdlička in the last lumbar vertebra, where the whole vertebra was divided into two parts. In such instances the separation usually occurs bilaterally, between the junction of the superior articulating processes of the vertebra and laminae. Manners-Smith and Els attribute the condition chiefly to mechanical factors, such as strain from flexion, occurring suddenly or of long duration. Hrdlička regards it as congenital. In osteological collections the posterior fragment is very easily lost, and thus a lumbar vertebra with a wide dorsal gap is encountered fairly frequently. A wide posterior gap, however, was not once observed throughout the 1,000 X-ray plates. Spina bifida of the other lumbar vertebrae does occur in varying degree, and records of such cases appear from time to time, usually in the clinical literature, but these are rather rare. A case of Voelcker (1903) is in point. Here, in a woman of 23, spina bifida of  $L_2$  is associated with a fatty and ligamentous tumor and various motor and sensory disturbances. George (1907) also gives a case of a child  $2\frac{1}{2}$  years old, where  $L_3$  and  $L_4$ , as well as the sacrum, are cleft in the midline and a dislocation of the left femur is present.

As has been said before, considerable clinical interest is attached to certain phases of lack of posterior vertebral union; *i. e.*, when it forms spina bifida occulta associated with neurological and other maldevelopments (Fuchs 1910, Krause 1911, Schulthess 1912, Els 1915, Findlay 1917). Among the maldevelopments, when the defect is in the lumbo-sacral region, may be mentioned trophic, sensory and motor disturbances of the lower extremities, bladder disturbances, uterine and rectal prolapse, club feet, congenitally dislocated hip, and pelvic and spinal malformations. Brickner (1918) has conveniently classified the abnormality into four divisions for clinical purposes: (1) Spina bifida occulta, with external signs and symptoms; (2) spina bifida occulta with external signs and no symptoms; (3) spina bifida occulta with no external signs but with symptoms; (4) spina bifida occulta with no external signs and no symptoms.

Of the 8 cases in this study in which the sacrum was found to be involved, 6 showed more or less marked clinical manifestations:

- L.E., male, 27 years. Sacrum only bifid. Extreme pain in back after working in stooped position. Slight scoliosis to left. No other suggestive physical findings.
- A.M., male, 23 years. Sacrum only bifid. Bladder paresis, involuntary voiding as a child, later incomplete emptying of bladder. Depression and blind sinus over sacrum. Rt. foot supinated and varus position; large trophic ulcer under proximal end of rt. 5th metatarsal.
- F.L., female, age 17 years, unmarried. Last lumbar and sacrum bifid. Congenital dislocation of right hip and well-marked congenital left-sided flat foot.
- M.R., female, age 55 years, nullipara. Sacrum only bifid. Pain in right leg and hip; pain in coccyx.
- S.R., female, age 35 years. Four healthy children.  $L_4$ ,  $L_5$ , and sacrum bifid. Hypertrichosis and naevus over sacral region. Marked lordosis. Rt. foot showed mild hallux valgus and Schaeffer's non-deforming club foot. Congenital relaxation of bladder sphincter (w. plastic operation which did not cure).
- E.S., female, age 26 years. Last lumbar bifid. Pain in lumbar region, began 1 year previous. Lumbar lordosis increased, and increased prominence of sacrum. Spine mobile. No tenderness.

Of the 15 cases (9 male and 6 female) in which the last lumbar vertebra only was involved, and the 5 cases (3 male and 2 female) in which the last lumbar and first sacral vertebræ showed defective ossification, only 1 case, and that in the former class, gave any clinical manifestations:

T.G., female, aged 36 years. Last lumbar bifid; lumbo sacral strain; pain in sciatic nerve. Retro-position of uterus.

In one instance in the group where the last lumbar showed an irregularity there were clinical manifestations of associated malformations:

C.T., female, aged 33 years. Two children, 14 and 12 years respectively. Last lumbar irregular; ureteral stricture left, and sacroiliac strain left side. Retro-position of uterus.

In the dorsal region incomplete posterior vertebral closure is very rare and, like those cases of the upper lumbar vertebræ, are only occasionally reported. In approximately 3,000 dorsal vertebræ seen at the National Museum the condition was not observed in a single instance. Joachimsthal (1895) presents the case of a "Woman with the horse's mane." In this there were evident interruptions in the second to the fifth vertebral arches, with hair 27 cm. long over the site of the defect. In the cervical region, other than with the atlas, a bifid condition of the vertebral arches is likewise rare, only one case being found among 3,500 cervical vertebræ examined at the National Museum. This was in the bones of a Massachusetts Indian (No. 227471), in which the fifth cervical lacked dorsal laminae. Unfortunately C<sub>4</sub> of the series was missing, nor were any other vertebræ present. These cervical vertebræ are shown in figure 6. One case was encountered when examining the X-ray plates; in this there were multiple spinal anomalies, and C<sub>7</sub> showed incomplete dorsal union:

E.S., male, age 16 yrs. In hospital for osteomyelitis of humerus. Right sided scoliosis in upper dorsal region. Body of D<sub>3</sub> replaced by two triangular wedges on right side and one on left. C<sub>7</sub> showed incomplete dorsal arch. Scoliosis with convexity to left in lumbar region.

Rauber (1907) cites a case in an adult male skeleton where C<sub>6</sub> showed a bifid condition. The case described by Barclay-Smith (1910) may also be mentioned here:

Young female Egyptian. Atlas synostosed to occiput. C<sub>2</sub> and C<sub>3</sub> fused together; C<sub>7</sub> interrupted neural arch dorsi-median; 2 laminae mutually independent, with spinous process subdivided; 8 cervical vertebræ and C<sub>8</sub> showed a small, unilateral cervical rib. L<sub>3, 4, 5</sub> and S<sub>1</sub> showed lateral interruptions to dorsal arch.

With the atlas the occurrence of a bifid condition of the vertebral arch once more appears as a fairly frequent variation. Here, as in the lumbar region, it is found in the vertebra which, in a freely articulating series, lies next to more rigid structures. Incomplete posterior arch of the atlas was observed in 11 out of 745 cases (1.47 per cent). Table 3 gives the incidence in the various groups studied. Two of these were included in a group of 50 cases of Swiss Alpine type, reported to me through the kindness of Dr. Adolf H. Schultz, and which belong to his private collection at Zurich. In addition to these 11 cases there are in Dr. Hrdlička's exhibit

cabinet at the National Museum 7 atlases showing incomplete posterior arch. The latter have not been included in the series, as they represent material which has been selected on account of this variation, while the 11 cases cited above belong to routine acquisitions. In 2 of the Indian specimens there was absence of practically all arch formation (figs. 7 and 8), while in all of the remaining specimens the two laminae approximate within 0.5 to 4 mm. of each other.

TABLE 4.

Classification.	Number of cervical vertebrae (excluding atlas).	Number of atlases.	Incidence bifid atlas.	
			Number.	Per cent.
Blacks:				
Melanesians.....	88	18		
Negriti, P. I.....	7	2		
Negro and mulatto.....	167	15		
Kaffirs.....	17	3		
Yellow-blacks:				
Lapps.....	33	6		
Chinese.....	12	2		
Eskimo.....	185*	32		
Indians.....	1662	349	5	1.43
Whites:				
Egyptians.....	425	126	1	0.79
Whites (miscellaneous).....	537	75		
Swiss (Alpine type).....		50	2	4.0
Miscellaneous, white and negro, J. H. U. Department of Anatomy.....	402	68	3	4.41
	3535	746	11	1.47

\*One case of incomplete posterior arch of C<sub>5</sub> (fig. 6).

Associated with this common type of incomplete arch there was a slight, though appreciable asymmetry in a little less than one-fourth of the specimens observed (4 out of 13 unbroken atlases, exhibit specimens included). This consists apparently in a bending of one of the lateral masses forward or backward, and sometimes also outward, with subsequent anterior or posterior projection of the corresponding dorsal lamina of the atlas, so that the two laminae did not meet symmetrically. Figure 9 represents an atlas of this type in the exhibit cabinet at the National Museum, while a symmetrical atlas is shown in figure 10, representing the Egyptian specimen designated in table 4. That this asymmetry is associated with an asymmetrical position of the condyles of the occiput seems probable, as the latter condition is found in all types of crania. It was impossible to follow up this point.

In attempting to analyze the various factors that seem to influence the closure of the posterior vertebral laminae, another group of cases, in which the atlas and occiput show physiological union, may be introduced. The condition has been frequently reported, and among 21 cases of physiological union found in the literature incomplete dorsal arch occurred 13 times, or 61.9 per cent, as shown in table 5.

In many of these cases a lateral twisting of the atlas on the occiput has been noted. At the National Museum there are 56 specimens of Dr. Hrdlička's collection showing fusion between the atlas and occiput. Probably in 6 of these the fusion was the result of a pathological process, and in none of them was the atlantal ring incom-



plete. Seven other specimens were so damaged that it was impossible to determine whether or not the atlas showed a bifid condition. In 43 intact specimens there was physiological union of atlas and occiput and, among these, incomplete union of the dorsal laminae of the atlas was found in 25 cases (58 per cent), the bifid condition (fig. 11) ranging from 1 to 12 mm., with an average of 4.16 mm. There was one exception (an Eskimo specimen) in which the dorsal laminae of the atlas were entirely lacking. In every case of bifid atlas there was a marked asymmetrical arrangement in its fusion with the occiput, one side being more closely united than the other. In the 18 cases in which the dorsal atlantal arch was complete there was a much greater degree of symmetry in fusion of the atlas to occiput, and in 14 of these this symmetry was very marked.

TABLE 5.

	Cases.	Open atlantal arch.
Dwight.....	1	1
Veraglia.....	3	2
Regnault.....	15	9
Le Double.....	2	1
	21	13

In summing up the various factors which may play an etiological rôle in these cases of minor incompleteness of the posterior vertebral arch, we must first take into account the residual influence of early embryonic spina bifida. We know that localized delayed closures in the embryonic central nervous system occur, and it is very likely that with some of the less severe types of this condition a moderate degree of lagging is set up, resulting in the incoördination of the growing parts, and finally in the lack of bony union of the vertebral dorsal arches. One would suppose this factor to be at work to a greater degree in cases with associated neurological, meningeal, or skeletal malformations, or where a considerable length of spinal column is involved, than in cases where only one vertebra shows the condition. Here other factors may be called upon to explain it. Thus, in addition to the embryonic theory, which undoubtedly brings us closest to thinking of the cause in chemical terms, owing to the knowledge we have of experimental NaCl spina bifida, we must consider also the mechanical theories that appear to contain rational suggestions. However, it must be borne in mind that with any biological theory there is no such thing as real separation of the physical from the chemical—they are always in closest association. While possibly mechanical factors are present in this condition, great care should be exercised in placing confidence in any unproved statements which they may represent, for here, especially, mechanical explanations are rather specious and almost impossible to test directly. The mechanical factor upon which most stress has been heretofore laid is that of longitudinal flexion of the vertebral column, and the point has been emphasized that it is in the region of greatest flexion that the bifid condition most frequently arises. The element of lateral torsion has been considered as only a possible, minor factor. It is possible, however, that this may be of importance, especially where its action in individual vertebrae would seem to be combined with factors such as the following: (1) Position of the last vertebra of a free series, lying next to more rigid structures; *i. e.*, atlas or last lumbar vertebra. (2) Asymmetry; *i. e.*, atlas opposed to asymmetrical condyles of occiput, or asymmetrical fusion occurring between atlas and occiput.

In several adult cases reported by Els a history of symptoms of spina bifida, appearing after more or less protracted trauma, is recorded. It is quite probable that in adults mechanical agents act more frequently as secondary than as primary etiologlcal factors, producing a clinical spina bifida, as differentiated from a solely anatomical condition. In early childhood, however, the possibility of such factors being primary must be taken into account, as the vertebral dorsal arches are at that time incompletely ossified.

We are not as yet far enough advanced to determine definitely the correlation between time and etiology in such a variation as is here dealt with, but it is hoped that a small amount of speculation, in an effort to supply a preliminary orientation in regard to this factor, will be pardoned. It would seem that during prenatal life the organism is more sensitive to metabolic disturbances, and that these would have greater effect upon form than similar factors acting at a later time. On the other hand, the individual would appear to be slightly more subject to mechanical disturbances, secondary, perhaps, to metabolic disturbances, resulting in slight changes of normal growth sequences, during early postnatal than in either prenatal or adult life. So, while one can not make positive statements as to either the time or the character of the disturbances producing the different types of spina bifida, there is the possibility, when the defect is a limited one, that its presence may have been partially due to mechanical factors occurring during childhood. Where the process is more extensive, however, such a possibility disappears, and in these there must have been a more fundamental metabolic disturbance acting at an earlier date.

Before closing, mention should be made of the fact, brought out by Frets, that in six-segmented sacra the percentage of lower closures ( $S_1$ ) of the hiatus of the sacral canal is greater (34 per cent) than in those with 5 segments (5 per cent); and that the percentage of reduction in the posterior arch of  $S_1$  is somewhat over (21.5 as opposed to 34.0) in 5-segmented than in 6-segmented sacra. Evidently the caudal extent of the sacrum is associated with a corresponding tendency towards posterior closure of the vertebral arches in this region—the longer the sacrum, the lower the closure of the hiatus.

#### SUMMARY.

The condition of incomplete closure of the vertebral posterior arches is present in the different regions of the spinal column in the following order of frequency:

	Cases.	Per cent.
(1) First sacral: Adolphi 12 per cent, Frets 26 per cent, author 13.1 per cent.		
(2) Entire sacrum:		
Radlauer.....	500	5.0
Adolphi.....	292	3.08
Frets.....	750	2.0
Frets.....	528	1.7
Frets (Breslau Anatomy).....	257	3.5
Average.....		2.89
(3) Last lumbar: author, 2.3 per cent in 1,000 cases.		
(4) Atlas: author, 1.47 per cent in 745 cases.		

5



6



7



8



9



10



11

FIGS. 5 and 6. Two specimens of the atlas in which there is incomplete dorsal arch formation. Fig. 5, specimen No. 258637 F2, U. S. National Museum. Fig. 6, specimen No. 262993, U. S. National Museum.

FIG. 7. Specimen showing symmetry of the dorsal laminae. Specimen No. 256457, U. S. National Museum.

FIG. 8. Specimen showing asymmetry of the dorsal laminae. Specimen No. 271782, U. S. National Museum.

FIG. 9. Posterior view of the cervical vertebrae, of which the fifth lacks dorsal laminae. Specimen No. 227471, U. S. Nat. Mus.

FIG. 10. Specimen showing open sacral canal and partial sacralization of fifth lumbar vertebra, the dorsal arch of which is bifid.  $\times 0.7$ . Specimen No. 17980506, U. S. National Museum.

FIG. 11. Specimen showing fusion of atlas and occiput, the posterior arch of the atlas being incomplete.



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CONTRIBUTIONS TO EMBRYOLOGY, No. 31.

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THE ARRANGEMENT AND STRUCTURE OF SUSTENTACULAR CELLS  
AND HAIR-CELLS IN THE DEVELOPING ORGAN OF CORTI.

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With four plates.

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## CONTENTS.

	PAGE.
Introduction.....	111
Methods.....	112
Historical.....	112
Connections between the outer supporting and hair-cells.....	116
First stage of development.....	117
Second stage of development.....	119
Third stage of development.....	124
Connections between the inner hair-cells and their sustentacular elements.....	125
Significance of some of the so-called cells of Hensen.....	128
Mitochondria and other structures in hair and supporting elements.....	129
Hair-cells.....	129
Supporting cells.....	132
Summary.....	135
Bibliography.....	139
Description of plates.....	141
General abbreviations.....	142



# THE ARRANGEMENT AND STRUCTURE OF SUSTENTACULAR CELLS AND HAIR-CELLS IN THE DEVELOPING ORGAN OF CORTI.

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BY O. VAN DER STRICHT.

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## INTRODUCTION.

The arrangement of the outer sustentacular and hair-cells of the organ of Corti in adult mammals is rather well known. According to the investigations of Held (1902), N. Van der Stricht (1908), and Kolmer (1909), the body of the cell of Deiters is situated below that of its sensory element, so that the two are connected by a chalice-shaped, greatly modified segment, in the concavity of which lies the deep cytoplasmic portion of the supported hair-cell. In the embryonic stages, however, the relation between these two elements is entirely different, and it might be well worth while to trace accurately their connections through the whole developmental stage. The same holds good for the inner and outer rods of Corti, the inner supporting cells, and even some of the so-called cells of Hensen.

Most investigators who have tried to clarify the arrangement of the sensory and sustentacular elements in embryonic material have made use of and describe vertical radial sections of the organ of Corti. Although very interesting, and in fact highly necessary, such preparations are liable to be deceptive and lead to misinterpretation. Indeed, most authors incorrectly represent the cells of Deiters. Many authors, even Retzius (1884) and Held (1909), who describe the phalanx process of the sustentacular elements as running obliquely from the cell body towards the lamina reticularis, thus crossing two or three hair cells, generally picture it in illustrations of vertical radial sections as an uninterrupted band connecting the nucleated portion of the cell with the free surface of the epithelium. So, also, do most authors of text-books of histology, notwithstanding the fact that in a radial vertical section this protoplasmic strand shows at least three interruptions.

By making use of sections tangential and always somewhat oblique to the surface of the organ of Corti, N. Van der Stricht was able to accurately locate the nucleated body of the supporting elements in successive stages of development and to determine the amount of gradual shifting. In this study the same method of research was applied in order to locate the more superficial portions of these cells between the sense-epithelium elements, and to ascertain their exact relation to the hair-cells and the mechanical factors that cause the shifting of the sustentacular elements. Moreover, a series of preparations, exhibiting mitochondrial structures in the supporting cells and hair-cells, has rendered it possible to define the nature of coarser structures noted by previous observers.

## METHODS.

Kittens, dogs, and rabbits, from birth to 12 days old, white rat fetuses, and young white rats about 2 days old, constituted the material used in these investigations. The isolated cochlea was fixed by trichloroacetic acid (5 per cent in water), by Bouin's or by Zenker's fluid, and subjected for many weeks to the mordant action of some drops of iodine in alcohol (70 per cent). Where necessary, after fixation by Bouin's or by Zenker's fluid, decalcification was completed by 2 per cent nitric acid in 70 per cent alcohol. Before embedding in paraffin the pieces were stained with borax carmine and the sections with iron hematoxylin, Congo red, and light green.

The mitochondria in the sustentacular and hair-cells were fixed in the following manner: Mixture of formalin and bichromate of Regaud (1910), according to the modifications indicated by Cowdry (1916), and subsequent staining with acid fuchsin and methyl-green; treatment by a 1 per cent aqueous solution of osmic acid for about an hour, followed by immersion in trichloroacetic acid, or Bouin's or Zenker's fluid; exposure of the cochlea, the bony wall of which had been previously provided with two or three small openings, to vapors from a 2 per cent aqueous solution of osmic acid for approximately 30 to 60 minutes, and subsequent treatment of the piece by one of the three above-mentioned agents; fixation for an hour in a 1 per cent aqueous solution of osmic acid, followed by immersion in a 1 per cent aqueous solution of silver nitrate for 3 hours. By these methods of fixation, and staining with iron hematoxylin and Congo red, the mitochondria can be brought into prominence within one or two turns of the cochlea, occasionally throughout its extent. Osmic vapors have been recommended as a fixing agent for mitochondrial structures by M. R. Lewis and W. H. Lewis (1914). We are able to confirm this statement, having for many years successfully used these vapors, and a subsequent treatment by another reagent, for the purpose of fixing the chondriomites in the ova of the dog. Henneguy (1895) was able to bring into view chondriocents in the spermatocytes of *Helix* by the use of osmic vapors.

The description given herein is illustrated by figures representing three different series of sections:

(1) Radial, vertical sections of the organ of Corti. These are cross-sections of the rows of hair and supporting cells, the knife cutting the latter along their length and from the axis toward the outer wall of the cochlea (figs. 14, 15, and 16).

(2) Spiral, vertical sections of the organ of Corti, these being longitudinal sections of the parallel spiral rows, the knife cutting the hair and supporting cells along their length, from the more apical to the more basal part of the spiral organ (figs. 19, 20, 22, and 23).

(3) Sections tangential and always somewhat oblique to the surface of the organ of Corti, the knife cutting transversely the hair and sustentacular cells of the spiral parallel rows at all levels, from the surface of the epithelium towards the basilar membrane (figs. 2 to 13, 17 and 18), so that their arrangement and structure can be traced in cross-sections throughout their lengths (figs. 2 and 3).

## HISTORICAL.

The structure of the organ of Corti has been exhaustively studied. It is known to be made up of two kinds of cells, the hair-cells and the supporting elements,

arranged in spiral, parallel rows: an inner row of inner hair cells on the medial side of the inner rods of Corti, and three rows of outer hair-cells on the lateral side of the outer pillars. According to Waldeyer (1872), Retzius (1884), Tafani (1884, 1885), who examined *Cercopithecus viridus*, and Kolmer (1910), four and occasionally five rows of outer hair-cells exist in man and monkeys; in other mammals a fourth row may appear in some parts of the cochlea. The hairs upon the free surface of all these elements were first described by Deiters (1860), and all authors agree that these sensory epithelial cells are cylindrical in shape and contain a single rounded nucleus in the deeper cytoplasmic portion. Deiters (1860), Hensen (1863, 1873), Middendorp (1867), Loewenberg (1868), Boettcher (1869, 1872), v. Winiwarter (1870), Krause (1876), and Nuel (1878), believed that the cell body of the sensory elements is connected with the basilar membrane by an intermediate deep process. That no such process exists has been conclusively proved by Rosenberg (1868), Retzius (1884), Denis (1901), Vernieuwe (1905), and by all of the more recent observers. All of the other elements of the organ of Corti are held to be supporting cells, and among these two types must be distinguished:

(1) *The two rows of inner and outer pillar cells or rods of Corti:* With the exception of Loewenberg (1868), who finds more outer than inner pillars, most investigators—Claudius (1855), Boettcher (1856, 1859), Max Schultze (1858), Middendorp (1867), v. Winiwarter (1870), Krause (1876), Nuel (1878), and many others, compute about three inner pillars for two outer; while N. Van der Stricht (1908) proves that the number of inner pillar cells is just double the number of the outer pillar cells, of the cells of Deiters of the first and second rows, and of the hair-cells of each row. Tafani (1884) had already noted that the number of outer pillars is exactly the same as the number of hair-cells of each outer row. He stated also that the phalanx apex of the outer pillars is located between two apices of neighboring hair-cells of the first outer row. Many misinterpretations have been published about the development of the pillars. Rosenberg (1868), Boettcher (1869, 1872), and Pritchard (1878) assert that one original cell divides into two, an inner and an outer pillar; whereas Loewenberg (1868), Gottstein (1870), Waldeyer (1872), and Hardesty (1915) regard each pillar as derived from two cells. The investigations of Hensen (1863, 1871), Middendorp (1867), Retzius (1884), Denis (1901), Vernieuwe (1905), and other more recent observers, have proved conclusively that each pillar with its nucleus is originally developed from one cell (the pillar cell). The striated fibrillar structure of the pillars in the adult cochlea has been noted by M. Schultze (1858), Boettcher (1859, 1869), Deiters (1860), Loewenberg (1868), v. Winiwarter (1870), Gottstein (1870), Hensen (1871), Nuel (1872, 1878), Lavdowsky (1876), Retzius (1884), and others; but their actual filamentous structure and the basal body of the outer pillars have been clearly brought out by Joseph (1900), Retzius (1900), v. Spee (1901), Held (1902), N. Van der Stricht (1908), and Kolmer (1910).

(2) *The outer supporting cells, or the cells of Deiters:* According to the investigations of all observers, these cells, like the pillars, are stretched between the basilar membrane and the lamina reticularis. They are composed of a nucleated cell body

and a more superficial portion, the phalanx process, the former running from the basilar membrane to the nucleated part of the hair-cell, the latter inclosed within the region of, and between the acoustic elements. Gottstein (1870), Waldeyer (1872), Nuel (1872), and Lavdowsky (1876) describe a true fusion of the apex of the nucleated part of the supporting element with the lower portion of the corresponding hair-cell, so as to produce a "twin or double cell." This view is to be regarded as a misinterpretation, and from the investigations of Tafani (1882, 1884), Retzius (1900), Held (1902), N. Van der Stricht (1908), and Kolmer (1910), it is plain that in adult animals a close connection exists between these two elements, the lower pole of the hair-cell occupying a cup-shaped depression in the upper pole of the supporting cell body. The wall of this pit is made up of a system of deeply staining fibrils which form a goblet- or chalice-shaped covering (Held) to sustain the sensory epithelial cell. This chalice extends down into a broad, fibrillar filament running obliquely toward the inner side of the nucleus; and coursing through the medial portion of the cytoplasm to reach the base of the cell, where the filament enlarges into a conical, fibrillated foot, the base of which rests upon the basilar membrane. At the level of the nucleus the filament divides into two branches, one of which is connected with the chalice, the other more slender one extending throughout the conical phalanx process of the cell of Deiters, to abut against the membrana reticularis. Parts of this filament have been noted by many previous observers. Its basal portion has been erroneously interpreted by Boettcher (1869, 1872), Lavdowsky (1876), and Nuel (1878) as a deep process given off from the neighboring hair-cell. The chalice and the branch of the filament beneath it were not observed by Retzius (1884, 1900), nor by v. Spee (1901), but these authors nevertheless describe a single fibrillar band extending throughout the cell body and its phalanx process. The chalice itself was first noted by Kishi (1902, p. 177), as "an dem unteren Ende der Haarzellen befindliches Gebilde von Kelchförmiger Gestalt," but misinterpreted as a nerve ending.

The real seat of the nucleated body of the cell of Deiters is illustrated and described by all authors in vertical radial sections, during the earliest stages in the development of the organ of Corti, as alternating with that of the more superficial hair-cells. Thus the supporting cell of the first row lies below an interstice separating the acoustic elements of the first row from those of the second; the supporting cell of the second row lies below an interstice separating the acoustic elements of the second row from those of the third; and the supporting cell of the third row lies outside the acoustic elements of that row. No alteration in the relation of the supporting elements to the hair-cells is mentioned. Even when representing each cell of Deiters beneath its respective acoustic element, Retzius (1884, plates xxii and xxiii), and many others, make no allusion to it in their descriptions. N. Van der Stricht is the only writer who has given an accurate explanation of these mutual relations, which can be seen in sections tangential and somewhat oblique to the surface of the epithelium, the two kinds of cells being represented by long, nucleated columns, the real position of which can not be misinterpreted. According to his investigations three successive stages are distinguishable:

(1) Outside the row of inner pillar cells there exists "une seconde rangée nucléaire à noyaux plus irréguliers, siégeant directement sous la rangée des premières cellules acoustiques externes; elle correspond aux noyaux des cellules à piliers externes [p. 580]. . . . Primitivement, les éléments de Deiters de la première rangée siègent nettement sous les cellules acoustiques externes de la seconde rangée, ceux de la seconde rangée sous les cellules acoustiques externes de la troisième rangée, ceux de la troisième rangée en dehors de la troisième rangée des cellules acoustiques externes." (2) "Plus tard, à mesure que l'organe de Corti évolue, il s'opère un refoulement des éléments de soutènement de dehors en dedans vers l'axe du limaçon. La rangée nucléaire des piliers internes est refoulée sous celle des cellules acoustiques internes. La rangée nucléaire des piliers externes est refoulée en dedans de la première rangée sensorielle externe. La première rangée nucléaire de Deiters atteint l'interstice séparant les deux premières rangées sensorielles externes. La seconde atteint l'interstice séparant les deux dernières rangées sensorielles externes. La troisième rangée nucléaire de Deiters est disposée à peu près sous la dernière rangée sensorielle externe [p. 666]. . . . (3) Les cellules de Deiters, après avoir subi un premier refoulement de dehors en dedans, amenant leur corps cytoplasmique en dedans de la rangée sensorielle, dans la quelle elles sont primitivement intercalées, en subissent un second dans le même sens, amenant le corps cellulaire directement sous celui de la cellule sensorielle voisine, de sorte que l'élément de Deiters, faisant partie primitivement de la seconde rangée sensorielle externe, siège définitivement sous une cellule de Corti de la première rangée sensorielle. L'élément de Deiters de la seconde rangée sensorielle arrive sous une cellule de Corti de la deuxième rangée sensorielle et l'élément de Deiters, siégeant primitivement en dehors de la troisième rangée sensorielle, est refoulé sous une cellule de Corti de cette dernière série." [p. 670].

Held (1909), commenting upon the paper of N. Van der Stricht, says (p. 243):

"Siene reichen Einzelangaben über die Entwicklung der Sinneshaare, der Lamina reticularis, der Stützfasersysteme kann ich zum grossten Teil bestätigen. Womit ich nicht übereinstimme, will ich im folgenden kurz hervorheben."

Although no objections are found in his succeeding pages against the theory of shifting of the supporting cells, described by N. Van der Stricht, Held does not recognize at all the three stages just mentioned. Indeed, referring to the close connection between the acoustic and supporting cells he says (p. 215):

"Die allgemeine Stellung der Deitersschen Zellen zu den äusseren Haarzellen wird während dieser Prozesse in keiner prinzipiellen Weise geändert."

The superficial phalanx process of the cells of Deiters, as first pointed out by Hensen (1863), reaches the corresponding phalanx interpolated within the lamina reticularis. In adult mammals it has been described by Nuel (1878), Retzius (1884, 1900), Tafani (1884), v. Spee (1901), Held (1902), and N. Van der Stricht (1908), as running obliquely and decussating with three, or even with four or five (v. Spee) hair-cells.

Many discordant theories have been advanced regarding the part played by the two epithelial thickenings of the cochlea duct in the development of the organ of Corti. Boettcher (1869), Hensen, Baginsky (1866), and Hardesty (1908) regard the lesser ridge as the germ from which all the supporting and hair-cells arise. Prentiss (1915) believes that the inner supporting cells, and probably the inner hair-cells and inner pillars are derived from the greater ridge, and that the lesser epi-

thelial thickening forms the external portion of the spiral organ. But at present no doubt exists as to the origin of these structures. According to Rosenberg (1868), Gottstein (1871), Boettcher (1872), Retzius (1884), Denis (1901), Vernieuwe (1905), N. Van der Stricht (1908), and Held (1909), the inner hair-cells originate from the greater ridge, all the pillars and outer hair and supporting elements from the lesser ridge; Rosenberg, Vernieuwe and Held locating the inner rods of Corti in an interstice outside the greater ridge between the two epithelial thickenings.

The superficial membrana reticularis of the organ of Corti has been referred to in a previous paper (1918).

It is evident from the above review that most of the structures of the spiral organ are at present well known. Nevertheless, many others require further investigation, and of these, four types will be dealt with herein, *i. e.*:

(1) The connections between the outer supporting cells and the hair-cells. If the view taken by N. Van der Stricht be correct, and the nucleated body of the sustentacular cell undergoes gradual shifting, what happens to its more superficial segment? What is its exact location during the three successive stages of development? What mechanical factor causes this alteration in the position of the cell body? At what period of development, and in what manner, does the spiral shifting of the apical process of the cell of Deiters take place?

(2) The connections between the inner hair-cells and their sustentacular elements.

(3) The significance of some of the so-called cells of Hensen.

(4) The nature and origin of peculiar, coarse structures in the cytoplasm of the hair and supporting cells.

#### CONNECTIONS BETWEEN THE OUTER SUPPORTING AND HAIR-CELLS.

In the new-born dog the epithelium of that part of the spiral membranous duct which lies close to the apex of the cochlea is still undifferentiated and is composed of elongated columnar cells, the apices of which reach the surface. A section tangential to these free ends shows a regularly formed mosaic of small, undifferentiated polygonal fields, each of which contains a diplosome. The polygons are separated from one another by terminal bars (referred to in a previous paper, 1918). On tracing the pattern through a series of sections, more and more remote from the summit of the cochlea, different structures are successively met with. First, within the greater ridge appear the inner hair-cells, recognizable by the enlargement of their cell bodies and nuclei, while in a section tangential to the surface their apices are seen in the form of rounded, sensorial fields, quite different from the neighboring supporting and non-differentiated polygons. Somewhat farther from the apex of the cochlea the outer hair-cells become differentiated and constitute the lesser epithelial thickening; there is an increase in the cytoplasm and the size of the nucleus, and the latter, like that of the inner sensory cells, stands out prominently in the vicinity of the epithelial surface, whereas the nuclei of the future supporting elements persist near the basilar membrane.

## FIRST STAGE OF DEVELOPMENT.

In figure 2 is illustrated a section tangential to the surface of the apical or first turn of the cochlea in a dog 12 hours after birth. Between the inner and outer hair-cells is seen a column of distinct, nearly square fields—cross-sections of a series of inner pillar cells (ip). The lowermost third of this column is nucleated and belongs to six pillars cut at six different levels. From mutual compression, together with an enlargement of this basal portion of the cell, the nucleus and its surrounding protoplasm are somewhat flattened out in a radial direction. The upper two-thirds of the column becomes gradually smaller and contains a darker, axial, granular strand. The clear superficial field of the column—the true apex of the cell—is the narrowest and incloses a central corpuscle. From this it is clear that the inner rod of Corti is a columnar cell, a four-sided prism in shape, enlarged at its base and tapering to its free surface. In more advanced stages (figs. 3 and 4) the enlargement and flattening of the basal portion of the cell are more marked, the apical portion thinner, while the intermediate, true cytoplasmic part increases in size.

Close to this spiral row of inner pillar cells, and situated within the greater ridge, may be seen (figs. 2, 3, and 4) cross-sections of three different rows of cells; one row of inner hair-cells (ih), and two rows of inner supporting cells ( $is^i$ ,  $is^{ii}$ ). The former extend through the superficial two-thirds of the epithelium and possess a dark, granular, and considerably increased cytoplasm with large nuclei in the lower portion of the cell. At the free surface of the cell is an eccentric, central corpuscle surrounded by a clear area—the medullary zone of the attraction sphere of Ed. Van Beneden, and a deeply staining plate—the superficial cuticula or the cuticular plate (N. Van der Stricht), from which the hairs arise.

Just below the cell bodies of the inner hair-cells lies the basal portion of the inner sustentacular cells of the first row (figs. 2, 3, 4 and 10,  $is^i$ ), composed of a smaller nucleus and a clearer protoplasm, which reaches the surface of the epithelium by means of a long cytoplasmic process, running between and compressed by the enlarged hair-cells. Being a four-sided prism at the level of its nucleated basal part, this columnar cell becomes lamellar, flattened out in a radial direction at the level of the acoustic elements (figs. 2 and 10), and by compression between two neighboring cells is pushed toward the inner pillars, its form being triangular on section, at first near the nucleated portion (figs. 2 and 3), and later also near the superficial cytoplasmic part of the inner hair-cells (fig. 4). Thus it is clearly seen that the inner supporting cells of the first row do not run laterally, either inside or outside of the inner hair-cells, but course through an interval between two acoustic elements, and by enlargement of the developing hair-cells are pressed towards the outer or lateral part of this interstice to take again their original position just beneath the superficial membrana reticularis.

The inner supporting cells of the second row (figs. 2 and 3,  $is^{ii}$ ) are four-sided prisms, composed each of a small basal nucleus and a clear cytoplasm. These cells undergo no compression from intermediate cells, but form a distinct spiral row, constituting the inner boundary of the organ of Corti, just as the cells of Deiters of the outer third row (figs. 2 and 3,  $d^{iii}$ ) constitute the outer limit of the spiral organ.



Outside of the inner pillars the organ of Corti, in the earliest stages of its development, when the outer superficial sensory fields (fig. 10,  $oh^i$ ,  $oh^{ii}$ ,  $oh^{iii}$ ) have attained about half the size of the inner (fig. 10,  $ih$ ), is formed of four spiral rows (figs. 2, 3, and 10): (1) The first row of outer hair-cells and outer pillar-cells ( $oh^i$ ,  $op$ ); (2) the second row of outer hair-cells and cells of Deiters of the first row ( $oh^{ii}$ ,  $d^i$ ); (3) the third row of outer hair-cells and cells of Deiters of the second row ( $oh^{iii}$ ,  $d^{ii}$ ); (4) the cells of Deiters of the third row ( $d^{iii}$ ). The outer hair-cells correspond with the inner in structure, except that their development and enlargement begin somewhat later, and that even in the adult cochlea their size is smaller. The outer pillar-cells and the cells of Deiters of the first and second rows are similar to the inner supporting cells of the first row in number, arrangement, and structure. Their cytoplasm is clear, and their nuclei occupy the basal one-third of the cell.

In sections tangential to the free surface of the epithelium these nucleated portions of the supporting cells are juxtaposed into nuclear columns, and, like the nuclear column of the inner sustentacular elements of the first row, lie directly beneath the acoustic cells of their respective columns. The superficial two-thirds of these outer supporting elements reach the membrana reticularis, running through the interval between two developing hair-cells by which they are compressed, so that the original four-sided prism (fig. 10,  $op$ ) becomes flattened out in a radial direction (fig. 3,  $op$ ,  $d^i$ ,  $d^{ii}$ ), and assume a lamellar, pentagonal (fig. 3) or triangular (fig. 2) shape on section. As the result of mechanical pressure from the neighboring hair-cells, which gradually increase in size chiefly in their nucleated portion, the superficial cytoplasmic processes of the outer pillars and the cells of Deiters of the first and second rows are compressed and pushed toward the medial side of their respective spiral rows—that is, toward the inner pillars, the superficial portion of which is thereby flattened out. The subsequent shifting of this compressing process becomes conspicuous, first at the level of the enlarged nucleated parts of the hair-cells (fig. 3,  $op$ ,  $d^i$ ,  $d^{ii}$ ), afterwards in the more superficial region (fig. 2).

This gradual, mechanical shifting of the upper two-thirds of these supporting cells ultimately results in their transposition into a system of new spiral rows or spaces as follows: (1) A spiral row of outer pillar cells situated between the spiral row of inner rods of Corti and the first row of outer hair-cells. This is illustrated in figure 4, showing the upper part of the outer pillars pushed inward from their original spiral row. This apical portion is increased in size and pentagonal in shape on section, and its outer angle still encroaches upon the original row, mainly close to the surface where it is connected with the phalanx or the apex of the outer pillar-cell, always interpolated within the primitive first outer spiral row. (2) A first outer interstice situated between the first and second rows of outer hair-cells (fig. 4,  $d^i$ ). (3) A second outer interstice situated between the second and third rows of outer hair-cells (fig. 4,  $d^{ii}$ ). The first and second outer interstices contain respectively the phalanx processes of the cells of Deiters of the first and second rows. These processes for a time may encroach upon their original sensory row, but later are entirely incorporated within their ultimate interstice except close to



the surface, where they join their apex, the stationary phalanx, interpolated in the lamina reticularis.

The cells of Deiters of the third row (figs. 2 and 3,  $d^{iii}$ ) are four-sided prismatic elements. In tangential section their nucleated basal portions form a nuclear column situated obviously outside of the third outer sensory row, and their superficial cytoplasmic processes are seen as a column of clear, superposed fields often somewhat flattened by the enlarged neighboring hair-cells. In figure 2 ( $d^{iii}$ ) their number is apparently the same as that of the cells of Deiters of the second row, but in the bat, according to N. Van der Stricht, there are two cells of Deiters in the third row for each one in the second row. Views from the surface of the membrana reticularis, as illustrated in figs. 10, 13, and 18, enable one to compute the apices of these cells ( $d^{iii}$ ), and prove that the cells of Deiters of the third row, if not double in number, are at least always more numerous than those of the second or first row.

The above-described seven spiral rows of the organ of Corti in the earliest stage of development can be distinctly seen in views of the surface of the membrana reticularis. As illustrated in figure 10, the arrangement of the apices of all the supporting and hair-cells is indisputably along seven spiral rows, each sharply demarcated. These rows are of two different types. The second row of inner sustentacular cells ( $is^{ii}$ ), the row of inner pillars ( $ip$ ), and the third row of Deiters cells ( $d^{iii}$ ), are purely sustentacular in character; while the others—the inner ( $ih$ ,  $is^i$ ), and the three outer rows ( $op$ ,  $oh^i$ ;  $d^i$ ,  $oh^{ii}$ ;  $d^{ii}$ ,  $oh^{iii}$ ) are mixed rows, supporting and sensory in character. At a slightly more advanced stage of development, however, this primitive condition changes (figs. 3 and 4), the structures assuming a definite arrangement (fig. 18) except for the apices of the pillar-cells, as seen in figure 13. The chief transformation, as compared with figure 10, consists in the appearance between the three mixed spiral outer rows of two interstices belonging entirely to supporting fields. The first is composed of parts of the apices of two varieties of cells, alternately the outer and the inner extremities of the phalanges of the respective outer pillars ( $op$ ) and the cells of Deiters of the first row ( $d^i$ ); and the second interval is of a similar pattern, alternately the inner and outer extremities of the phalanges of the respective cells of Deiters of the first and second rows. The sensory fields are not altered. The supporting fields, or the phalanges, undergo an elongation and extend over the two interstices appearing in the depth of the epithelium. The apices of all the cells remain *in situ*, but the phalanges become elongated and cover parts of two developing supporting interstices.

#### SECOND STAGE OF DEVELOPMENT.

This stage is characterized chiefly by the shifting of the nucleated portions of the outer pillar and outer supporting cells inward and toward the axis of the cochlea, so that, as shown in figures 5, 6, and 7, the basal part of the outer pillars ( $op$ ) is found to be entirely inside of the first row of outer acoustic elements; that of the first row of Deiters cells ( $d^i$ ), inside of the second row of acoustic elements; that of the second row of Deiters cells ( $d^{ii}$ ), inside of the third row of acoustic elements; and that of the third row of Deiters cells ( $d^{iii}$ ) either partially or almost entirely beneath

the third row of acoustic elements. The basal, nucleated portion of these supporting cells follows the shifting of their more superficial part, and each of the three originally mixed outer spiral rows divides into two distinct rows: One, purely sensory, which does not reach the basilar membrane; the other, purely sustentacular, which is continuous throughout the thickness of the epithelium. Of all these elements the apices alone maintain their primitive position. At the same time other important changes are occurring. The basal, nucleated portion of the cells of Deiters undergoes a slight enlargement, while the increase in the superficial cytoplasmic part is inconspicuous; as a result of mutual compression between the neighboring hair-cells it is still flattened out in a spiral direction and thereby reduced in size.

In this respect the outer pillars differ from the outer supporting cells. Like the inner pillars they enlarge rapidly, and three parts, without marked outlines, become distinguishable; *i. e.*, a nucleated basal portion, or foot, which is the largest; an intermediate, cytoplasmic portion, of medium size, which tapers off to a more superficial portion, the latter being the smallest (fig. 5). At its foot the outer pillar-cell assumes the form of a four-sided, and in its more superficial part a five-sided pyramid (figs. 4 and 5), the basal part of which is flattened out in a radial direction (fig. 5, op). A similar but more pronounced increase in size and a subdivision into three segments are noticeable in the inner rods of Corti (figs. 4 and 5, ip), the foot and nucleus being markedly flattened out in a radial direction. Moreover, the foot extends considerably toward the foramina nervina, seemingly repelling the bases of the neighboring inner cells. This process of extension has been pointed out by many observers—Hensen, Boettcher, Middendorp, Retzius and others. No true shifting of the base of any supporting cell, however, occurs. The expansion of the foot of the inner pillars inward and of the foot of the outer pillars outward is accompanied by a corresponding extension of the subjacent basilar membrane. This has already been emphasized by Vernieuwe and others, and should be attributed to abundant nutriment from the neighboring *vas spirale*. This elongation of the base of the inner pillar-cell is accompanied by an alteration in the inclination of the inner pillar itself, and causes likewise an alteration in the inclination of the inner supporting and hair-cells. The degree of slant of these cells is difficult to determine accurately, although it is quite marked. Indeed, sections tangential to the surface of the organ of Corti, in the earliest stages of development, represent cross-sections through all of its elements (figs. 2, 3 and 4). When the inner pillars extend toward the foramina nervina such sections cut transversely the components of the outer part of the spiral organ (figs. 6, 7, 8 and 9), while the inner pillars, the inner hair and supporting cells are frequently cut along their length. This can be explained only by their marked inclination outward and towards the surface, and the inclination of the outer hair and supporting elements in another direction, so that the axis of the inner supporting cells (fig. 16, *is*<sup>i</sup>) meets that of the outer at nearly right angles.

During the second stage of development of the organ of Corti, and soon after the protoplasmic portions of the cells of Deiters reach their neighboring interstice, another remarkable shifting occurs—a shifting in the spiral direction of the organ,

as illustrated in figs. 19, 19<sup>i</sup>, 20, 21, and 23. These are diagrams of vertical spiral sections through the outer spiral rows. In figures 19 and 19<sup>i</sup> there can be seen to the left a more basal portion of the second turn of the cochlea in a new-born kitten, and to the right a more apical portion, consisting of the following elements: A series of acoustic cells of the first outer row (oh<sup>i</sup>) recognizable by the presence, between the apices of the hair-cells of a bundle of fibrils (op) emanating from the heads of the outer pillars; a series of acoustic elements of the second outer row (oh<sup>ii</sup>), and a series of hair-cells of the third row (oh<sup>iii</sup>). The clearer intervals filled with longitudinal tangential sections through the lateral surfaces of acoustic elements, and situated between the first and second, between the second and third row, and at the end of the third row (compare with figs. 20 and 21) are respectively the first, second, and third outer sustentacular interstices.

Below these sensory elements are seen three series of the nucleated portion of the cells of Deiters of the first (d<sup>i</sup>), second (d<sup>ii</sup>) and third (d<sup>iii</sup>) rows; these are connected with the membrana reticularis by a thin, superficial phalanx process, running obliquely through their respective interstices (fig. 20, d<sup>i</sup>, d<sup>ii</sup>). In the three turns of the cochlea in the kitten, young dog, and young rabbit these apical processes always course regularly below upward, and from a more basal to a more apical portion of the turn, crossing the medial side of three hair-cells (figs. 19, 21, oh<sup>ii</sup>, d<sup>i</sup>; oh<sup>iii</sup>, d<sup>ii</sup>), or the lateral side of the hair-cells of the third row (fig. 19<sup>i</sup>, oh<sup>iii</sup>, d<sup>iii</sup>).

From the above description it is clear that during the second stage of development the cells of Deiters undergo a shifting in two directions—one axial, from without inward; the other spiral, from the base of the cochlea upward and toward the more apical portion of it. What mechanical factors are involved in this process? The chief framework around which the constituents of the organ of Corti are built up is undoubtedly the original single spiral row of inner pillar-cells (fig. 2, ip). Very early this framework becomes stronger, owing to the appearance of an additional row of outer pillars (figs. 4 and 5, ip, op); and although the inclination of these two kinds of rods of Corti is liable to change, the axis of the framework (figs. 1, 3, 4, 5, and 14), even when the tunnel space is present (figs. 15, 16, and 17,  $\tau$ ), is more or less perpendicular to the basement membrane and passes through a part of the lumen of the vas spirale (vs). As development progresses, this spiral framework becomes more and more wedge-shaped or triangular in sections, the summit of the triangle being formed by a very narrow field—the apex of the inner pillar (figs. 2, 5, and 15, ip), so long as the tunnel space is not developed, and the broad base of the triangle consisting of the adjoining feet of the inner and outer rods of Corti. As the result of this rapid and considerable extension of the base, the deepest part of the cells of Deiters, although immobile on the basilar membrane, is removed farther from the axis of the framework. On the other hand, by comparing the illustrations of the membrana reticularis in figures 4, 10, and 18, the lateral extension of this is rather inconspicuous and the apices of the outer supporting cells do not follow the lateral shifting of their bases. The result is that the direction of the nucleated supporting columns seen in figures 2, 3, and 4, (op, d<sup>i</sup>, d<sup>ii</sup>, d<sup>iii</sup>), lying just beneath their respective columns of supported sensory elements (oh<sup>i</sup>, oh<sup>ii</sup>, oh<sup>iii</sup>) will change, their bases

resting upon the membrana basilaris, and farther removed from the axis of the framework than their more superficial portion in connection with the lower pole of the hair-cells. Due to this pressure from the axial framework, and especially from the feet of the outer pillars, the nucleated portion of the cells of Deiters tends to follow an oblique direction upward and inward. In other words, the superficial part of the nucleated cell body will tend to join the contiguous spiral interstice into which its phalanx process has already been projected during the first stage of development (fig. 5, op,  $d^i$ ,  $d^{ii}$ ) and the nucleated part of the cells of Deiters of the third row will be pushed beneath the lower pole of the hair-cells of the third row (fig. 5,  $d^{iii}$ ).

Another mechanical factor plays an important part in this shifting of the outer supporting cells. On enlarging and elongating, the outer hair-cells not only expel from their spiral row the supporting elements and efface the intercellular spaces left by them, but they also extend laterally so that each sensory row becomes thicker, particularly at the level of the nuclei, while the diameters of the apices of the hair-cells do not change during the second stage of development. This broadening of the three outer sensory rows results in a more obvious enlargement of this part of the lesser ridge, which in turn causes a bulging towards the outer wall of the cochlea (figs. 5 and 15), since the presence of the tough axial framework renders expansion towards the axis impossible. Hence the outer hair-cells run obliquely (fig. 14), downward and outward. While the upper pole of the nucleated portion of the cells of Deiters tends to turn inward, the lower pole of the sensory elements tends to turn outward, so that the axis of the interstices between the sensory rows gradually blends with that of the basal portion of the supporting elements (fig. 6). This result is obtained by the concomitant action of a third mechanical factor, the elongation of the hair-cells, and probably by the elongation of the cells of Deiters. In figure 5 the nucleated columns ( $d^i$ ,  $d^{ii}$ ) do not encroach upon the more superficial interstices, while in a more advanced stage (fig. 7) they obviously invade the lower region of these spaces. This invasion is caused primarily by the elongation of the hair-cells between the subjacent cell bodies of the supporting elements. No true intercellular spaces exist at the level of the latter; nevertheless between them are found spiral nerve bundles ( $N^{iii}$ ,  $N^{iv}$ ,  $N^v$ ) connected with the lower pole of the hair-cells by ascending branches. Such a disposition is, of course, apt to facilitate the invasion of these intercellular nerve spaces by the elongating sensory elements. The explanation of the shifting of the phalanx processes of the cells of Deiters in a spiral direction and toward the apex of the cochlea appears to be more difficult. Before attempting any interpretation it must be borne in mind that the extremities of these elements, the basal resting upon the membrana basilaris, and the superficial interpolated in the membrana reticularis, always remain fixed. Of course these membranes may increase in size, regularly or irregularly, so that a point  $A$  upon the former, just beneath a point  $A^i$  upon the latter, may, by a process of unequal development, become situated just below a point  $B^i$  or  $D^i$ , the point  $A^i$  being removed farther from basal part of the cochlea; but point  $A^i$  will never take the place of point  $B^i$ , such as has already been proved is the case with portions of the cell bodies

that are pushed out from a mixed spiral row, or from beneath the hair-cell, into a new space. If this view is correct it must be recognized that the membrana reticularis undergoes a process of extension somewhat different from that of the membrana basilaris. It is a well-known fact that the development of the spiral organ of Corti proceeds from the base of the cochlea towards its apex and the sense epithelium may be well developed in the third turn, while in the second it is still in process of differentiation, and as yet undifferentiated in the first. Moreover, it has already been mentioned that during the first stage of development the growth and enlargement of the hair-cells, hence of the superficial two-thirds of the epithelium, are more marked than that of the cell bodies of the sustentacular elements constituting the basal third of the epithelium. From this it is evident that the former, and its superficial membrana reticularis, extend more rapidly than the latter, not only towards the lateral side of the organ of Corti, but also in a spiral direction from the third turn toward the second, and from the second toward the first; that is to say, from points where the pressure is high to places where it is lower. According to this principle it is not surprising that during the first stage of growth of the organ the apices of the Deiters cells precede their bases in extending toward the apex of the cochlea, so that the deeper part of the cell remains behind three hair-cells, crossed by the phalanx process.

The second stage of growth, characterized by the peculiar arrangement of the cells of Deiters, lasts a long time, and many other important transformations occur before the final, third stage is reached. The tunnel space between the rods of Corti may appear, but it is noteworthy that the definite arrangement of the outer supporting cells, which is peculiar to the third stage, may be attained before any formation of a tunnel space (fig. 9).

The most important role in the production of the third stage of development is played by the cells of Deiters, chiefly by their extension in length. During the first and the beginning of the second stage the sensory elements undergo a rapid increase in size and attain their full volume. Afterwards the nucleated body of the Deiters cell becomes gradually longer, as illustrated in figures 7, 20, 19, 19<sup>i</sup>, 14, 15, and 16 (d<sup>i</sup>, d<sup>ii</sup>, d<sup>iii</sup>). In earlier stages (figs. 1, 2, 3 and 4) their nuclei, like those of the rods of Corti (op, ip) are situated close to the basilar membrane, and the nucleated cell body is very short. Later (fig. 19) it gradually extends and soon attains about the length of the acoustic elements (fig. 21, d<sup>i</sup>, d<sup>ii</sup>, d<sup>iii</sup>), its nucleus becoming farther removed from its base (figs. 19 and 19<sup>i</sup>, d<sup>i</sup>, d<sup>ii</sup>, d<sup>iii</sup>), reaching its top rather abruptly (figs. 20, 21, d<sup>i</sup>, d<sup>ii</sup>, d<sup>iii</sup>). Finally, when the nucleated body of the Deiters cell attains two-thirds (figs. 16, 15, d<sup>i</sup>, d<sup>ii</sup>, d<sup>iii</sup>) or more (fig. 23, d<sup>i</sup>) of the thickness of the epithelium, its superficial cytoplasmic end appears to lengthen out more rapidly than its deeper parts and to give rise to a very important segment of the cell, which, for the sake of its physiological function, may be termed the *supporting segment*, within which a peculiar framework or sustentacular apparatus makes its appearance, the nucleus occupying about the middle (fig. 16) or lower part (fig. 23) of the superficial one-third of the cell body.

## THIRD STAGE OF DEVELOPMENT.

This is characterized by the peculiar arrangement of the cells of Deiters. Instead of being situated between the interstices of the rows of outer acoustic elements, the body of each sustentacular cell lies just beneath that of its respective supported hair-cell, so that each acoustic element of the first, second, and third rows is supported respectively by the body of a cell of Deiters of the first, second, and third row, as shown in figures 8, 9, 11, and 17 ( $d^i$ ,  $d^{ii}$ ,  $d^{iii}$ , and  $oh^i$ ,  $oh^{ii}$ ,  $oh^{iii}$ ). In other words, the axes of the nucleated columns of hair-cells, hence of each individual hair-cell, blends more or less (figs. 8 and 17), or even exactly (figs. 9 and 11), with that of the nucleated columns of supporting elements—that is, of the cells of Deiters.

In order to obtain a true picture of the mechanical factors which take part in the final arrangement of the sustentacular elements it is only necessary to examine and compare figures 14, 15, and 16, especially the latter, representing the period of rapid elongation of the body of the Deiters cell. In figure 14 the supporting segments ( $d^i$ ,  $d^{ii}$ ) embrace one half of the lower pole of each neighboring hair-cell; in figure 16 they show varying connections with these poles which can only be ascribed to their varying capacity for extension. Owing to the peculiar disposition of the elements of Deiters and the obliquity of their axes, the elongation of the cell body and its segment of support is possible in one direction only—*i. e.*, towards the pole of the hair-cell which will eventually be supported by it. Indeed, on lengthening out the segment,  $d^i$ , impinges upon  $oh^{ii}$ , and from this pressure the inclination of the latter may be more pronounced; but the extension of  $d^i$  proceeds towards and around the outer part of the lower pole of  $oh^i$ , so that ultimately the axes of the acoustic elements of the first row will blend with those of the cell bodies of the sustentacular elements of the first row (figs. 8 and 17,  $oh^i$ ,  $d^i$ ), and the axes of the hair-cells of the second and third rows will blend respectively with those of the cell bodies of the sustentacular elements of the second and third rows (figs. 8 and 9,  $oh^{ii}$ ,  $d^{ii}$ ;  $oh^{iii}$ ,  $d^{iii}$ ). It may be mentioned that during this process of shifting, the spiral nerve bundles, originally situated outside their respective rows of outer hair-cells (fig. 3,  $N^{iii}$ ,  $N^{iv}$ ,  $N^v$ ) are pushed inward, along with the neighboring supporting elements, and now occupy the interstice inside of these sensory rows (fig. 9,  $N^{iii}$ ,  $N^{iv}$ ,  $N^v$ ).

The upper portion of the body of the Deiters cell—that is, the apex of the segment of support, extending around the lower pole of the hair-cell—becomes converted into a cup-like depression which surrounds and enwraps the deeper cytoplasmic part of the acoustic element, particularly at its lower and outer portion, its inner portion for a time remaining free. Whereas in figure 9 this depression is still inconspicuous, in figure 11 (owing to a considerable enlargement of the segment of support) it is seen to be deeper and its margins separate the inclosed pole of the hair-cells from the neighboring elements except those situated inside of the pole. In a more advanced stage this denuded part of the hair-cell will in turn be surrounded by the expanding segment of support.

During these transformations there appears within the cell of Deiters a part of its apparatus of support, originally consisting of a bundle of fibrils, or a fibrillated

filament, extending uninterruptedly from the basilar membrane to the membrana reticularis. This filament rests upon the basilar membrane with a fibrillated triangular foot, and runs through the nucleated cell body in a direction parallel with the axis of the cytoplasm, but occupying the medial part of the latter. The filament is not *axial*, but *paraxial* (fig. 16, d<sup>i</sup>, d<sup>ii</sup>, d<sup>iii</sup>), and is in direct continuity with another, which occupies the axis of the cytoplasmic phalanx process (fig. 21, d<sup>i</sup>, d<sup>ii</sup>, d<sup>iii</sup>), and may be termed the *apical filament*. Whereas the paraxial filament originally (fig. 16) courses through the inner part of the body of the supporting element, curves about its apex, and continues along with the apical filament (fig. 21, d<sup>i</sup>, d<sup>ii</sup>), it later traverses the cell body obliquely, since above the nucleus (fig. 18, d<sup>i</sup>, fig. 8, d<sup>i</sup>, d<sup>ii</sup>, d<sup>iii</sup>), and in still more advanced stages at the level of the nucleus (fig. 9, d<sup>i</sup>, d<sup>ii</sup>), it blends with the axis, reaching the lateral part of the cytoplasm and extending into the apical process. This singular modification in the course of the paraxial filament at the level of the segment of support is undoubtedly due to the latter's peculiar process of development and furnishes striking evidence that it extends unequally (fig. 16, d<sup>i</sup>), as above mentioned; that is, more toward the hair cell supported by it in the third stage of development (oh<sup>i</sup>) than towards the one supported by it in the first stage (oh<sup>ii</sup>), hence more inwardly than outwardly.

The apparatus of support is completed by the appearance of a third fibrillated filament, the *axial* filament of the sustentacular segment of the cell of Deiters, expanding into a fibrillated, chalice-like enlargement which develops in the wall of the cup-shaped depression. The first trace of this axial filament and its apical chalice is illustrated in figure 11 (d<sup>i</sup>). On tracing the paraxial<sup>1</sup> filament of the cell of Deiters of the first row (d<sup>i</sup>), from the region of the nucleus towards the hair-cell (oh<sup>i</sup>), it is seen to divide into two branches just at the point where it reaches the axis of the cell. One branch (the outermost) runs outside the acoustic element and represents the apical filament; another very short branch (the innermost, visible in three cross-sections) is the axial filament which blends with and is replaced by a deeply staining semicircle, partially surrounding the cytoplasmic pole of the acoustic element (oh<sup>i</sup>) and imperfect on the inner side of the latter. This incomplete ring is the cross-section of an elongated, still imperfect goblet—the chalice in process of development. The cells of Deiters of the second and third rows also exhibit the apical filament, the axial filament, and the chalice (fig. 11, d<sup>ii</sup>, d<sup>iii</sup>). The apparatus of support is thus composed of a paraxial filament or stem, which divides into two branches, an apical filament and an axial filament with its chalice. Figure 23 shows parts of these structures (d<sup>i</sup>) at a more advanced stage when the sustentacular segment of the cell of Deiters has reached nearly its full extent and contains a much longer axial filament in continuity with the chalice.

#### CONNECTIONS BETWEEN THE INNER HAIR-CELLS AND THEIR SUSTENTACULAR ELEMENTS.

All authors agree that the greater epithelial thickening of the cochlea is made up of elongated, columnar cells, which undergo many changes and become converted, at the level of the sulcus spiralis, into a simple row of rather low, columnar



or cubical elements (fig. 15, esp), while close to the inner hair-cells a few retain their original, elongated form (figs. 15 and 17, nd), the latter being erroneously termed by some investigators, *inner supporting cells*. Indeed, they represent undifferentiated epithelial elements, and in the cochlea of most adult mammals their number is reduced to that of the true inner supporting cells. The character of these elements and the relation they bear to the neighboring sensory elements should be more accurately investigated. Most authors agree also that, during this stage of development, when the sulcus spiralis is being built up, many of the columnar cells undergo degeneration and disintegration, although very few observers are able to picture or even describe such a process. In the cochlea of the dog, cat, rabbit, and ox two distinct regions should be distinguished. One of these is medial, the true sulcus spiralis (fig. 15, esp), where not the slightest evidence of cell disintegration is ever seen, the high, columnar cells becoming gradually shorter, larger, and flattened out to cover the sulcus, which at first is narrow, but which subsequently, as the lumen of the duct enlarges, acquires considerable size. The other is lateral, just inside of the inner hair-cells (figs. 15 and 17, ih) or the foramen nervinum (N), where the columnar cells (nd) persist for a long time, and where occasional elements may undergo chromatolysis (fig. 17, eh), while most, if not all, of the others become converted into larger and shorter lining-epithelium cells.

External to, or even partly encroaching upon the foramen nervinum (figs. 15 and 17, N), are found the two rows of inner supporting cells already referred to as belonging to the first inner mixed and second inner supporting spiral rows. In the course of development the inner supporting cells undergo no marked shifting or cytoplasmic differentiation. In this respect they differ from the cells of Deiters, although resembling the latter and possessing many of the same essential characteristics; for example, (1) original connections with the hair-cells, (2) number, and (3) the shape of their free apices.

(1) The first inner row, like the three outer spiral rows, originally is a mixed row of sensory and sustentacular elements, and remains so, the supporting elements being entirely inclosed within the spiral row and running through the intervals between the hair-cells. Moreover, the free apex of the sustentacular elements, interpolated within the membrana reticularis, is more or less phalanx-shaped. The inner supporting cells of the second row, like the cells of Deiters of the third row, belong to a spiral row purely sustentacular in character; but whereas the latter, after a process of shifting, become transformed into a true sustaining framework for the neighboring hair-cells, the former preserve their original position. In other words, the two rows, the second inner and fourth outer, are originally boundary rows of the organ of Corti. The former does not change in its nature, but the latter develops into a true sustentacular row, at least as regards the cell bodies of its components.

(2) As to the inner supporting cells of the first row, no doubt can be entertained, the spiral row being composed of alternating sensory and sustentacular elements, and their number exactly the same as that of the supporting cells of each of the three



outer spiral rows. The number of cells of the two boundary rows is more difficult to determine, although in surface views of the membrana reticularis their free apices can be readily enumerated. In this respect the number of inner supporting cells of the second row (figs. 10, 12 and 13,  $is^{ii}$ ) corresponds to that of the neighboring acoustic elements ( $ih$ ), while in the cochlea of the bat, according to N. Van der Stricht, and also as illustrated in figure 10, the original number of apices of the cells of Deiters of the third row ( $d^{iii}$ ) is just double that of the neighboring hair-cells ( $oh^{iii}$ ), while in figure 5 ( $d^{iii}$ ) it is the same as the latter, and in figure 13 there are four sensory apices ( $oh^{iii}$ ) in contact with five sustentacular fields ( $d^{iii}$ ). On the other hand, in the adult bat there are just as many sensory apices as sustentacular fields; hence it is plain that in the course of development the number of cells of the outer spiral boundary row is reduced. Further investigation is required to clear up the significance of this reduction. It seems possible, even probable, that this row represents a fourth mixed spiral row, which in man and in some animals may become differentiated into hair and supporting cells.

(3) The apices of the inner sustentacular elements of the first row resemble those of the cells of Deiters of the first and second rows; they are also even more compressed between the apices of the inner acoustic elements, so that from being originally phalanx-shaped (figs. 12 and 18,  $is^i$ ) they become much thinner (fig. 13  $is^i$ ), and later are veiled by a deeply staining covering derived, according to previous investigations (O. Van der Stricht, 1918), from the surrounding terminal bars.

The apices of the sustentacular elements of the second inner row resemble those of the cells of Deiters of the third outer row. Indeed, in the first stage of development both are represented by polygonal fields (fig. 3,  $is^{ii}$ ,  $d^{iii}$ ) of about the same size, but the former ( $is^{ii}$ ) may be a little larger, particularly more elongated in a spiral direction (figs. 10 and 12,  $is^{ii}$ ) owing to higher pressure from the inner hair cells ( $ih$ ). In more advanced stages (fig. 13,  $is^{ii}$ ) they are represented by a very narrow, lanceolate field containing a central corpuscle and circumscribing the inner border of the acoustic elements. In the adult cochlea of the bat (*Vespertilio fuscus*, *Pipistrellus subflavus*), of the dog, and of the rat, this streak or line is covered also by a deeply staining veil produced by the terminal bars (the extension of the terminal bars over these fields is noticeable in figure 13,  $is^{ii}$ ), and the spiral row of apices of cells is then seen in the form of a dark homogeneous streak deeply stained by iron hematoxylin, which constitutes a very sharp demarcation between the membrana reticularis and the large polygonal fields (fig. 13,  $nd$ ) belonging to undifferentiated columnar cells.

The apex of the third row of cells of Deiters maintains more or less its original, polygonal shape (fig. 13,  $d^{iii}$ ); it covers a very dark triangular, fibrillated band or plate, the summit of the apical filament of the sustentacular element, as described by Held (1902) and N. Van de Stricht (1908).

Baginsky (1886) recognizes below the deep extremity of the inner hair-cells two other elements, one situated internal and the other external to the inner acoustic cell; both are connected with the surface, the former by a process running along the inner

side of the hair-cell, the latter along the outer side. This element, close to the inner pillar, should be regarded as homologous to the cell of Deiters.

The "inner phalanx cells," or supporting cells of the first inner row, with their superficial phalanx-shaped apices, are accurately described in the adult organ of Corti by Held (1902), who mentions in connection with the phalanx the existence of a plate or process extending from the head of the inner pillar between two neighboring apices of inner hair-cells. This plate ("Innensehnabel der Innenpfeilerzellen") has been observed also by Waldeyer (1872), Nuel (1878), Retzius (1885), and Kolmer (1909). In reality it does not exist, and what has been mistaken for it is probably only the dark, more superficial veil derived from the terminal bars.

According to N. Van der Stricht (1908), the nucleated bodies of the inner supporting cells, located in the greater ridge in the embryo bat, are found beneath the inner acoustic elements, and (p. 611) "de ce segment basal, renfermant le noyau, part un prolongement superficiel effilé, qui s'engage entre deux cellules acoustiques internes, pour atteindre la surface du neuroépithélium." This author did not recognize the shifting of the intermediate portion of their cytoplasm towards the inner pillars.

Referring to the development of the membrane of Corti, Held (1909, p. 219) mentions, inside of the row of inner phalanx cells, a row of "Grenzzellen, deren freie Zellenflächen die Köpfe der inneren Haarzellen an ihrem axialen Umfang umgreifen," but he does not give any further description of these elements. In his figure 18 Kolmer (1909, p. 309) pictures "doppelzeilige Stellung der inneren Haarzellen, Köpfe der Innenphalangen und Grenzzellen, mehrreihige Anordnung der Haare," but he, likewise, gives no further explanation.

#### SIGNIFICANCE OF SOME OF THE SO-CALLED CELLS OF HENSEN.

All observers are in agreement concerning the arrangement of the cells of Hensen, representing them as elongated, columnar elements, extending from the membrana basilaris to the free surface of the epithelium; but further investigation is necessary to prove the correctness of this statement. In the earliest stage of differentiation of the organ of Corti, at which time the apices of the outer hair-cells attain only half the size of those of the inner (fig. 2), there can be seen in sections tangential to the surface and outside the third row of Deiters cells, a column of elements exhibiting nuclei at two different levels; two near the membrana basilaris ( $ad^{iv}$ ), and two others nearer the free surface of the epithelium ( $aoh^{iv}$ ). The arrangement of these two sets of nuclei or nuclear groups is very remarkable; one ( $ad^{iv}$ ) runs parallel to the nuclear column of Deiters cells ( $d^{iii}$ ), the other parallel to the nuclear column of hair-cells ( $oh^{iii}$ ). This peculiar disposition would suggest that the deeper nuclei belong to the sustentacular elements and the more superficial nuclei to the acoustic elements, although there is no evidence of any differentiation into sensory cells at the level of their apices.

Similar structures are seen in later stages of development, and are all the more remarkable because here (fig. 5) the cytoplasm surrounding the nuclei has under-

gone a peculiar differentiation. Around the nuclei of the deeper group ( $ad^{iv}$ ) it appears clear or vacuolated like that of the cells of Deiters ( $d^{iii}$ ); around the nuclei of the superficial group ( $aoh^{iv}$ ) it is dark and granular, like that of the hair-cells ( $oh^{iii}$ ). From this it is plainly evident that the protoplasm of these two cells ( $ad^{iv}$  and  $aoh^{iv}$ ) is beginning to differentiate respectively into supporting and sensory elements; but their apices, as can be seen on close examination of serial sections, are represented by a mosaic of undifferentiated polygons similar in character to those of the true neighboring cells of Hensen. In reality, therefore, these cells should be regarded respectively as aborted supporting ( $ad^{iv}$ ) and sensory ( $aoh^{iv}$ ) elements of a fourth spiral row, which in man, especially, and also in some animals, become completely developed. In most animals, however, they retain their primitive character of simple, lining epithelial, or atrophied acoustic elements. Quite exceptionally a fifth spiral row of aborted hair-cells may be observed (fig. 7,  $aoh^v$ ) external to the fourth ( $aoh^{iv}$ ).

The atrophied acoustic elements are characterized by their arrangement into a spiral row situated external to the third row of cells of Deiters, and by the fact that their lower ends never reach the membrana basilaris (fig. 11,  $aoh^{iv}$ ). The apex of the cell is represented by a large polygonal field containing a diplosome (fig. 13,  $aoh^{iv}$ ), not unlike the apex of the true cell of Hensen. The atrophied hair-cells of the fourth outer row persist during the course of development and are illustrated in sections tangential to the surface in figures 8, 9, 17, and especially in figure 11 ( $aoh^{iv}$ ), and also in vertical, radial sections in figures 15 and 16 ( $aoh^{iv}$ ). The atrophy continues, apparently, for in adult animals these cells are much shorter than in embryonic stages, although they are never absent.

As regards the atrophied cells of Deiters (figs. 2 and 5,  $ad^{iv}$ ) these belong to the spiral row of atrophied hair-cells and, contrary to the normally developed sustentacular elements, do not reach the surface of the epithelium. As seen in vertical, spiral sections (fig. 21,  $ad^{iv}$ ), they alternate with the more superficial elements ( $aoh^{iv}$ ) without passing between them. The phalanx process is absent, so that at this point the epithelium is formed of two strata of atrophied cells. Further investigations will have to be made before the arrangement of the atrophied sustentacular cells in more advanced stages of development can be determined. The atrophy of these elements seems to be accompanied and caused by the lack of corresponding nerve fibers.

#### MITOCHONDRIA AND OTHER STRUCTURES IN HAIR AND SUPPORTING ELEMENTS.

##### HAIR-CELLS.

In the earliest stages of development of the organ of Corti (fig. 1) the cytoplasm of the acoustic elements is densely packed with mitochondria, which are lined up into granular chondriomites, or, as is usually the case, fused together into uniform filaments or chondrioconts. These are seen to run parallel with the axis of the cell and produce a longitudinal striation; in more advanced stages of development they still constitute the bulk of the protoplasm (figs. 19 and 20,  $oh^i$ ,  $oh^{ii}$ ,  $oh^{iii}$ ; fig. 9,  $ih$ ).

In transverse sections of the cells the cytoplasm appears granular, the filaments being cut across (fig. 9, oh<sup>i</sup>, oh<sup>ii</sup>, oh<sup>iii</sup>). Due to their presence, the protoplasm, even in specimens where the mitochondria are not stained, is always compact and dark. In the cochlea of the white rat, before birth and also two days after birth, these chondriosomes stained red by fuchsin and the nuclei green by methyl-green, after fixation by a mixture of formalin and bichromate.

Densely accumulated and packed chondriomites give rise to coarser structures, the so-called body of Retzius, the body of Hensen, and the superficial cuticula and hairs. The presence of the body described by Retzius (1884, p. 366) as "*eine grössere, körnig erscheinende Protoplasmaansammlung*," has been confirmed by Held (1902), N. Van der Stricht (1908), and Kolmer (1909), in the outer hair-cells. It exists within these elements in white rats before birth and in other new-born mammals (fig. 1, oh) in the form of a deeply staining granular mass of chondriomites, which incloses in its concavity the lower pole of the nucleus. In later stages of development (fig. 21, oh<sup>i</sup>, oh<sup>ii</sup>) the subnuclear, cytoplasmic portion of the hair-cells becomes much longer and larger, and near its lower extremity contains a rounded, not sharply marked-off mitochondrial body, formed of an outer zone of chondriosomes and a clear central fluid, in the center of which a coarse granule may be noticed. The body of Retzius is described by N. Van der Stricht in the cochlea of the adult guinea pig and bat (p. 653) as formed "*d'une couche compacte renfermant généralement un ou plus rarement deux corpuseles*." The significance of the central granule is uncertain, but the presence of such an accumulation of chondriosomes near the nucleus, and particularly at the lower pole of the cell, where the nutritive supply affects and penetrates into the cell body, seems natural and logical. Moreover, the inconstant occurrence of this body may be ascribed to a transference of its chondriomites toward the more superficial portions of the cytoplasm. In other words, it seems to be a source for developing mitochondria, whence they migrate into other parts of the cell.

The "Spiralkörper" of Hensen is described by Retzius (1884, 1900) as granular; it has also been observed by v. Spee (1901), Held (1902), N. Van der Stricht (1908), and Kolmer (1909). V. Spee regards this body as a transformed "central corpusele" surrounded by pigment granules. N. Van der Stricht considers it as a "centrosome" derived from an accessory central corpusele of the attraction sphere, this corpusele becoming shifted from the surface into the depth of the cell. The body of Hensen is not apparent in the earliest stages of development (fig. 1), but makes its appearance later in some of the outer acoustic elements beneath the free apex of the cell (fig. 20, oh<sup>i</sup>, oh<sup>ii</sup>, oh<sup>iii</sup>; fig. 19, oh<sup>ii</sup>). Like the body of Retzius, it is not a constant, permanent structure. It is essentially mitochondrial in nature, being formed of densely accumulated and packed chondriomites (fig. 20), and sometimes contains a kind of centrosome, a clear central area surrounding a special granule. This mitochondrial body should not be confounded with the long axial cone composed of closely arranged chondriomites or chondriocents, which is often visible between the apex and nucleus of the cell. The base of this cone contains the body of Hensen and is applied against the free surface of the cell.

The superficial mitochondrial body is closely connected with another allied structure—the superficial “cuticula”—from which the hairs arise, and which appears to be a center of mitochondria, developing probably around an accessory centrosome and capable of supplying the cuticula with chondriomites. The superficial plate of the acoustic elements from which the hairs arise is mentioned by Retzius (1884) as “ein ausserst dünnen Hautschiebt,” the inner surface of which is beset by “beim Kaninchen dicht, bei der Katze und dem Menschen mehr zerstreut, sehr feine, gleich grosse Körner” (p. 366). It is termed “Kopfeinlage” by v. Spee, “Haarplatte” by Held (1909), and “cuticula” by N. Van der Stricht. At the surface of the cuticula the latter author describes an “implantation plate or implantation crescent,” which gradually gives rise to a cone of fibrils or hairs. Although this crescent is closely connected with the diplosome, it is not formed by the central corpusele, but is derived from the cuticula. Hence the sensory cell is to be regarded as a ciliated but not a vibratile element. It is worthy of note that Fürst (1900), many years before, had seen at the surface of the hair-cells of the crista and macula acustica in an embryo salmon aged 90 days, a disc deeply stained by iron hematoxylin, which seemed to be composed of granules (“Basalkorperehen”), centrosomic in character, from which arose the cilia or hairs. Hence Fürst regards the hairs as specialized, differentiated vibratile elements.

In most preparations the superficial cuticula of the acoustic elements is seen in the form of a homogeneous plate which intensely stains dark blue, like the hairs, by iron hematoxylin (fig. 4, oh<sup>i</sup>, oh<sup>ii</sup>, oh<sup>iii</sup>, ih). On tracing the mitochondrial structures from the level of the nucleus toward the cuticula (figs. 4 and 9), the chondriomites are found to be more and more condensed and numerous, running close to the surface and giving rise to a superficial circular plate. This plate covers the largest part of the apex of the cell, leaving space within a notch on its lateral border for the diplosome (fig. 3). This mitochondrial cuticula usually appears homogeneous and uniform, although in some specimens obviously granular in structure (fig. 9, oh<sup>i</sup>, oh<sup>ii</sup>, oh<sup>iii</sup>), and in a few selected preparations the arrangement of the mitochondria into granular filaments (true chondriomites) is conspicuous. This is best illustrated by figure 12, showing a series of inner hair-cells (ih) cut transversely at successive levels near their apices, so that from right to left one sees three superficial granular crescents, or the cilia or implantation plates; three dark, circular, granular cuticulæ, with the neighboring central corpusele, and within the second cuticula three radiating chondriomites; three masses composed of still deeply stained, coarse granules, situated directly beneath the cuticula; and three finer, granular fields of finer mitochondria of a somewhat deeper portion of the superficial cytoplasm of the acoustic elements. Such figures justified the statement in a previous paper (1918, p. 63) that “this proves that cuticular formations belonging to the first series mentioned above (p. 57) may be of mitochondrial origin, but in addition it is a striking proof of the mitochondrial nature of the acoustic hairs formed by this plate.”

In other specimens where the mitochondria are also brought prominently into view by suitable fixing agents and stains, mainly by the mixture of formalin and

bichromate, and staining by fuchsin and methyl-green, the superficial cuticula appears homogeneous and, like the hairs, remains unstained. After fixation with osmic vapors or osmic acid, and staining with iron hematoxylin, pale, clear cuticulae may be observed. From these observations it would appear that the chemical constitution of the hairs and cuticulae is somewhat different from that of the original chondriosomes. Similar chemical changes have been noted in the development and evolution of mitochondrial structures. Without emphasizing those observed in gland-cells, it may be recalled that during the period of growth and genesis of the yolk of the ovum the pseudochromosomes (O. Van der Stricht, 1904), and the vitellogenic bands (1909), both mitochondrial in nature, undergo analogous alterations.

#### SUPPORTING CELLS.

From the earliest stages of their differentiation (fig. 1) the pillar-cells (ip, op), the inner (is<sup>i</sup>) and outer (d) sustentacular cells contain numerous chondriomites, and like the acoustic elements are longitudinally striated, owing to the longitudinal disposition of the chondriosomes. The young cells of Deiters (d) possess a mitochondrial body in close contact with the lower pole of the nucleus. In more advanced stages, before the appearance of coarser permanent structures, the arrangement of the chondriosomes differs according to the nature of the supporting element. In the inner sustentacular elements, within which no peculiar structures are formed, the chondriomites remain scattered more or less regularly throughout the length of the cytoplasm (figs. 9 and 15, is<sup>i</sup>).

As illustrated in figure 2, within the upper part of the pillar cells (ip, op) and the cells of Deiters of the first (d<sup>i</sup>) and second (d<sup>ii</sup>) rows, appears an axial, darker granular strand which is mitochondrial in nature (fig. 4), formed of closely arranged chondriomites. The band is surrounded by a clearer exoplasm within which the chondriosomes are less numerous. In figure 4 this mitochondrial strand reaches the base of all these sustentacular elements except that of the cells of Deiters of the third row (d<sup>iii</sup>), and the clearer cytoplasm becomes obviously reticulated or vacuolated, being pervaded by a network along which fewer mitochondria are scattered. In the meshes of the network are seen vacuoles formed of a pale, more fluid material. The mitochondrial bundle, longitudinally striated (figs. 15 and 9, ip) in vertical sections, occupies the portion of the protoplasm within which the supporting fibers will appear in a more advanced stage (fig. 8, ip), these filaments being produced by a fusion of the juxtaposed ends of the chondriomites.

In the inner pillar-cells the chondriomites become densely grouped around the axial strand at the level of the apical portion of the cytoplasm, so that on cross-section they form a mitochondrial ring or circle (figs. 3, 4, and 12, ip) inclosing the central part of the band, within which they are thinly scattered. This ring corresponds to the transverse section of a tube, the "Fibrillenröhre" of Held (1909), developed according to that observer (p. 209) "von der freien Seite her und von dem ganzen Rand und Umfang ihrer Schlussleisten." It would be more correct to say that this mitochondrial tube extends from the superficial centrosome towards

the deeper portions of the cell, where, at the level of the intermediate and basal parts it is converted into a solid latero-medial lamellar strand (figs. 3 and 4, ip) reaching the basilar membrane. In more advanced stages the tube itself becomes transformed into a solid, cylindrical (fig. 21, ip), and later into a flattened, lamellar mitochondrial bundle (fig. 18, ip) which also soon occupies the medial half of the superficial segment of the pillar (fig. 20, ip). This longitudinal, lamellar mitochondrial bundle, extending throughout the latero-medial portion of the protoplasm, is illustrated in vertical sections (figs. 15 and 9, ip), and by coalescence of its chondriocents gives rise to the fibrillar supporting apparatus of this inner pillar (figs. 14, 17, and 8, ip).

Besides this bundle, the bulk of the inner pillar during the second and third stages of development is formed, at the level of its basal and intermediate segment, of a clear, vacuolated cytoplasm (figs. 4, 5, 14, and 16, ip), occupying its axial and lateral portions. Its apical segment is composed of a compact, almost homogeneous protoplasm (fig. 5, ip) external to (fig. 20, ip) or even surrounding (fig. 18, ip) the supporting bundle.

The axial mitochondrial strand of the outer pillar-cells (fig. 2, op) also becomes converted into a paraxial or lateral, solid bundle, flattened or lamellar in shape, which originally runs more or less vertically throughout the three segments of the cytoplasm (figs. 4 and 5, op). In more advanced stages, while the apical segment undergoes an enlargement and develops the head of the pillar, the mitochondrial strand becomes obviously interrupted at the level of the junction of the two upper segments. The superposed chondriocents (fig. 20, op) fuse together and give rise to two distinct fibrillar bundles; a lower bundle, belonging to the basal and intermediate parts of the pillar (figs. 9, 14, 15, and 16, op), and a superficial one, belonging to the apical segment—that is, the fibrillar supporting band connecting the phalanx with the head of the pillar (fig. 13, op). This filamentous band, originally vertical, later runs obliquely towards its phalanx between the superficial cytoplasmic portions of two neighboring outer hair-cells (fig. 14, op), its course becoming more horizontal as development progresses (figs. 16 and 17, op). Thus, during the second and third stage in the development of the organ of Corti, the outer pillar, at the level of its basal and intermediate segments, is composed of a lateral mitochondrial or fibrillar, lamellar bundle, and an axial and inner, large, vacuolated portion (figs. 4, 5, 14, 16, 7, and 9, op), representing at a certain time (fig. 9, op) the bulk of the cytoplasm. At the level of its apical segment the pillar is composed of its oblique or nearly horizontal fibrillar bundle and a compact cytoplasm—the bulk of the head.

It is worthy of note that prolongations from the superficial terminal bars separating the apices of the inner and surrounding those of the outer pillars, and constituting part of the membrana reticularis (figs. 18 and 13, tb), extend between the future heads of the inner and outer rods of Corti and sever not only their adjoining surfaces (figs. 14, 15 and 16, tb), but also the contiguous surfaces of the heads of the two rows (fig. 9, tb). Under the influence of this modified cellular cement (the terminal bars) there appear to develop firm, compact bodies, originally



in direct contact with the bars, and described by Joseph (1900) as "Einschlüssen" of the heads of the inner and outer rods of Corti.

The supporting apparatus of the cells of Deiters, its stem or paraxial, fibrillar bundle, and its two branches—the apical filament and the axial strand with the chalice—is laid down by the original mitochondrial band extending throughout the length of the outer sustentacular elements (fig. 4,  $d^i$ ,  $d^{ii}$ ,  $d^{iii}$ ). The chondriomites of this strand are clearly illustrated in figures 9 and 19 ( $d^i$ ,  $d^{ii}$ ,  $d^{iii}$ ). By a process of coalescence of the chondriomites (fig. 10,  $d^i$ ,  $d^{ii}$ ,  $d^{iii}$ ) the fibrils of the apical (fig. 21) and of the paraxial, medial (figs. 25 and 16,  $d^i$ ,  $d^{ii}$ ,  $d^{iii}$ ) filaments are produced. The axial filament, with its chalice, appears in more advanced stages, when the segment of support of the cell of Deiters becomes enlarged and elongated below the hair-cell. At this time a long, axial, compact, and mitochondrial band is formed within the upper part of the nucleated cell body (fig. 23,  $d^i$ ), and by accumulation and coalescence of the chondriomites the short axial filament, along with its superficial chalice, is built up (fig. 11,  $d^i$ ,  $d^{ii}$ ,  $d^{iii}$ ). The chondriosomes of this axial part of the segment of support of the sustentacular elements have been described by Retzius (1900, p. 80), as "eine schwarz gefärbte Körneransammlung," which do not represent centrosomes and bear no relation to filaments. After examining these preparations M. Heidenhain stated that the granules may be either multiple, central corpuscles, or structures analogous to the "Basalfilamente" in other cells.

Besides their supporting apparatus, the cells of Deiters are composed of a much more abundant, clear, and often distinctly vacuolated (figs. 5, 7, 9, and 11) cytoplasm, which is less conspicuous within their phalanx process, although recognizable in some preparations (figs. 5 and 14). The paraxial filament always occupies the medial half of this vacuolated protoplasm (fig. 16,  $d^i$ ,  $d^{ii}$ ,  $d^{iii}$ ), and the latter becomes reduced around the axial band and its chalice (fig. 23,  $d^i$ ), when these structures make their appearance.

From the above description it is clear that the fibrillar framework of the rods of Corti and of the cells of Deiters appears in the earliest stages of development and is mitochondrial in nature. Similar filamentous structures have been described in lining epithelium cells by Firket (1910), Favre and Regaud (1910), and Sakae Saguchi (1913). The fibrillated apparatus and vacuolated cytoplasm of the sustentacular cells seem to be related to two different functions of these elements, the former to an absolutely conspicuous supporting function, the latter to a nutritive function. In order to get a true picture of the capacity of nutrition of the rods of Corti and the outer supporting cells, we should bear in mind that during the earliest stages of development of the organ the epithelial constituents undergo a rapid enlargement. Peculiar and complex structures—the superficial cuticula with its hairs, the membrana reticularis, and a part of the membrana tectoria—spring from the surface of the neuro-epithelium, which is destitute of blood-capillaries. There exist no intercellular channels to convey the nutritive material from the subjacent vascular tissue of the membrana basilaris to the superficial portions of the epithelium. Minute intercellular spaces undoubtedly exist, and their cement gives



rise to the thick, firm terminal bars which close the spaces and separate them from the endolymph of the cochlea duct. By close examination of figures 3, 4, 5, 7, 9, and 11, it is obvious that the vacuolization of the sustentacular elements becomes more pronounced as the basilar membrane is approached, and that the chief supporting cells—the inner and outer pillars—at first undergo considerable enlargement and acquire a markedly vacuolated structure quite close to the subjacent vas spirale (vs). which rapidly increases in size along with the transformations just described. Later, when the definite structures of the organ of Corti are nearly built up, the vas spirale becomes reduced in size (figs. 4, 16, and 15, vs). These facts furnish abundant evidence to support the theory that the fluid contained within the meshes of the network, or the contents of the vacuoles within the cytoplasm of the supporting elements, is derived from the blood of the vas spirale by means of a process of exudation through its endothelial wall and the adjoining basement membrane. This food supply, stored in the cytoplasm of the sustentacular cells, may undergo further glandular or chemical alteration before being utilized in the building up of the apparatus of support of the rods of Corti and the cells of Deiters, and transferred to the neighboring hair-cells.

#### SUMMARY.

1. The first stage in the development of the organ of Corti is characterized by the existence of seven sharply marked, spiral rows of cells. Of these the inner pillar cells and two boundary rows—*i. e.*, the second inner row of supporting elements and the third row of Deiters cells—are purely sustentacular in character. The remaining four are mixed rows, being composed of alternating sensory and sustentacular elements, and constituting the row of so-called inner hair-cells and the three rows of so-called outer hair-cells. The hair-cells extend through the superficial two thirds of the epithelial layer, and are separated from each other by the cytoplasmic portions of their respective sustentacular elements, the basal, larger, nucleated part of which fills the space between the hair-cells and the basilar membrane, as illustrated in figure 22 (*is*<sup>i</sup>, *ih*). In sections tangential to the surface each of the four mixed rows is seen in the form of two nucleated columns; one, the column of exclusively supporting elements, near the basilar membrane; the other, the column of alternating sensory and sustentacular elements, near the surface of the epithelium.

2. The original four mixed spiral rows are formed as follows: (*a*) The row of inner hair-cells is composed of inner acoustic elements and inner supporting elements; (*b*) the first row of outer hair-cells, of outer acoustic elements and outer pillar-cells; (*c*) the second row of outer hair-cells, of outer acoustic elements and the first row of Deiters cells; (*d*) the third row of outer hair-cells, of outer acoustic elements and the second row of Deiters cells.

3. The superficial cytoplasmic two-thirds of the supporting cells of the mixed spiral rows undergoes compression from the rapidly enlarging neighboring acoustic elements. This pressure results in a flattening and subsequent shifting of these portions of the inner supporting cells towards and into the lateral part of the spiral

row, and of those of the outer sustentacular elements towards the axis of the cochlea and into interstices between the primitive rows; so that three new spiral rows or spaces make their appearance. Indeed, each original mixed spiral row becomes divided into two—a medial or inner supporting column, and a lateral, purely sensory column. Hence the first mixed spiral row of outer hair-cells is divided into a column composed of parts of outer pillars and a column of acoustic elements; the second mixed spiral row of outer hair-cells is divided into a column composed of parts of the cells of Deiters of the first row and a column of acoustic elements; the third mixed spiral row of outer hair-cells is divided into a column composed of parts of the second row of Deiters cells and a column of acoustic elements.

4. The original membrana reticularis is formed of seven very distinct spiral rows of fields, the apices of the subjacent cells, which are separated from one another by terminal bars. When each of the three outer mixed spiral rows becomes double near the surface of the epithelium, two spiral intervals appear within the lamina reticularis, one between the apices of the hair-cells of the first and second rows, and the other between the apices of the acoustic elements of the second and third rows, these intervals being covered by portions of the neighboring elongated phalanges.

5. A fundamental principle governs the arrangement of the supporting elements during the earliest stages of development of the organ of Corti. By compression from neighboring constituents the cell body may become shifted, but the base, which is attached to the membrana basilaris, and the apex interpolated within the membrana reticularis, remain fixed. These may enlarge with the extension of the membranæ, but there is never any shifting.

6. The second stage of development is characterized by the shifting of the nucleated cell bodies of the outer supporting elements from their original spiral row; that is, from beneath the hair-cells of that row, inward and to a point just below the superficial, supporting interstices appearing in the first stage of development; so that the latter now constitute complete spiral rows extending throughout the thickness of the epithelium, whereas the three original mixed outer rows are converted into a purely sensory one, occupying the superficial two-thirds of the epithelium. External to the inner pillars, from the axis of the cochlea towards the outer part of the lesser ridge, there exist the following spiral rows: One of outer pillars, the first row of outer hair-cells, the first row of cells of Deiters, the second row of outer hair-cells, the second row of cells of Deiters, the third row of outer hair-cells, and the third row of cells of Deiters, the nucleated cell bodies of which show a tendency to shift below the neighboring acoustic elements.

7. The shifting of the nucleated portions of the outer supporting cells is the result of several mechanical factors: (a) The appearance of an axial framework within the organ of Corti represented by two kinds of rods of Corti. By the basal extension of this framework the bases of the cells of Deiters are pressed outward much more than is the apical portion of their nucleated cell bodies. (b) The broadening and lengthening of the outer hair-cells, which is possible only towards the outer side of the lesser ridge. The summit of the nucleated portion of the

supporting elements is, therefore, repelled inward and ultimately incorporated into and completing the superficial sustentacular interstices, which already contain the phalanx processes of the supporting elements. (c) This shifting is facilitated by the previous transference of the phalanx processes and by the peculiar connections between the lower pole of the hair-cells and the subjacent spiral nerve bundles.

8. Pressed out from their original mixed row, and having reached their respective interstices, the phalanx processes of the first and second rows of Deiters cells, together with those of the third row, undergo a shifting in a spiral direction, their apices moving from a basal to a more apical portion of the cochlea, so that they cross three hair-cells in their course towards the membrana reticularis. Whereas the acoustic elements follow a nearly vertical course, the cell bodies, and particularly the phalanx processes of the cells of Deiters, run more obliquely (figs. 19, 19'). This spiral shifting is due to an unequal development or extension towards the apex of the cochlea of the two membranæ to which are attached the two extremities of the cells of Deiters, the membrana reticularis extending more rapidly than the membrana basilaris.

9. The third stage of development is preceded by a considerable elongation of the nucleated cell bodies of the elements of Deiters and the appearance of a peculiar portion—the segment of support or sustentacular segment. On lengthening out obliquely from the nucleated part of the cytoplasm towards the lower pole of the hair-cell which will eventually be supported by it, the apex of the cell body enlarges and extends into a long segment surmounted by a cup-like depression surrounding the lower pole of the acoustic element; so that the axes of the hair-cells of the first, second, and third spiral rows ultimately blend respectively with those of the cells of Deiters of the first, second, and third rows.

10. Lateral to the spiral row of inner pillars the final structure of the organ of Corti is composed of the following supporting and mixed spiral rows: The row of outer pillars; the first mixed row of superficial acoustic elements supported by the subjacent bodies of the cells of Deiters; the superficial interstice containing the phalanx processes of the Deiters cells of the first row; the second mixed row of superficial acoustic elements supported by subjacent bodies of the cells of Deiters; the superficial interstice containing the phalanx processes of the cells of Deiters of second row; the third mixed row of superficial acoustic elements supported by the subjacent bodies of the cells of Deiters; and the superficial interstice containing the apical processes of the cells of Deiters of the third row.

11. At the third and final stage of development the spiral nerve bundles between the cells of Deiters, which originally were situated lateral to the primitive outer mixed rows (fig. 3,  $N^{iii}$ ,  $N^{iv}$ ,  $N^v$ ) occupy a place inside of the mixed spiral rows (fig. 9,  $N^{iii}$ ,  $N^{iv}$ ,  $N^v$ ).

12. The supporting apparatus of the cells of Deiters is originally formed of a stem or paraxial fibrillated filament, running straight throughout the inner portion of the prismatic cell body, at the apex of which it curves around and continues as an oblique apical fibrillar filament in the phalanx process of the cell. In later stages

of development the summit of the cell body becomes lengthened out into its segment of support; the paraxial filament, at the level of the nucleus, courses obliquely through the lateral part of this segment and gives off another branch—the axial filament—thus expanding into a fibrillar chalice located within the wall of the cup-shaped depression.

13. Contrary to the opinion of many authors, the columnar epithelium of the greater epithelial thickening of the cochlea duct does not undergo disintegration during the development of the sulcus spiralis. At the level of the latter no cells are lost, but all become flattened out to cover the greatly extending furrow. Near the organ of Corti the columnar cells persist longer, and occasionally exhibit evidences of chromatolysis.

14. Medial to the inner mixed row of hair-cells there exists a second row of inner sustentacular elements with differentiated apices. These resemble in many respects the cells of Deiters of the third outer row, although in the course of development the cell bodies of the latter are shifted beneath and support the neighboring acoustic elements. Like the sustentacular cells of the first inner row, those of the second remain in their original row; the apices of both take part in the formation of the membrana reticularis, that is, the apparatus of support of the apices of the hair-cells. Hence they represent true sustentacular elements. Like the cells of Deiters of the third row, the sustentacular elements of the second row also represent boundary elements.

15. Among the so-called cells of Hensen there exists, lateral to the third row of cells of Deiters, a fourth outer, mixed row, formed of alternating atrophied sustentacular cells and more superficial hair-cells. The atrophied hair-elements become shorter in the course of development, but persist, even in the adult cochlea. As a rule, the absence of nerve fibers seems to result in atrophy of the constituents of this fourth row, but in man these become normally developed.

16. From the earliest stage of development the cytoplasm of the hair and supporting cells contains innumerable chondriomites and chondrioconts which, by their peculiar arrangement, give rise to a longitudinal striation. By their aggregation the chondriosomes form the body of Retzius and the body of Hensen, each one representing a center of developing chondriomites, whence the chondriomites migrate into other portions of the cell. The superficial body of Hensen is closely connected with the cuticula of the hair-cell. This superficial plate is derived from coarse, coalescing chondriomites which appear to undergo a chemical alteration. Hence the hairs arising from the cuticula should be regarded as of mitochondrial origin.

17. The outer and inner rods of Corti and the cells of Deiters contain a fibrillated framework which, from the earliest stages of development, appears in the form of mitochondrial bands or strands. Each fibril is the result of fusion of the superposed chondrioconts. In addition to the sustentacular function of the framework, these elements possess also a function of nutrition. Indeed, the bulk of their cytoplasm is clear, and contains, besides a few chondriosomes, a serous fluid which exudes from the subjacent vas spirale and permeates the protoplasm of the cells in

the form of clear vacuoles. The number, size, and distinctness of these vacuoles decrease progressively from the base of the supporting element towards the surface of the epithelium, where important phenomena of metabolism and elaboration of peculiar structures take place, not only within the sustentacular elements, but also within the hair-cells and at the surface of the sensory epithelium.

The present investigation was started in the anatomical laboratory of the Medical School of the Western Reserve University, Cleveland, Ohio. It is my agreeable duty to express my sincere and profound gratitude to Professor T. Wingate Todd and for the gracious hospitality afforded me, and for all the material, reagents, and instruments which he placed at my disposal.

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## DESCRIPTION OF PLATES.

All figures were outlined with a Zeiss camera lucida at the level of the stage of the microscope, with the aid of ocular No. 3 and 2 mm., homog. immersion. Apert. 1.30.

### PLATE 1.

- FIG. 1. Radial vertical section of organ of Corti through the apical part of the cochlea. Dog 18 hours after birth. Fixation: osmic acid, trichloroacetic acid. Stain: iron hematoxylin and Congo red.
- FIG. 2. Section tangential to surface of organ of Corti through first turn and near apex of cochlea. Dog 12 hours after birth. Bouin's fluid, iron hematoxylin, and Congo red.
- FIG. 3. Section tangential to surface of organ of Corti through first turn of cochlea. New-born dog. Trichloroacetic acid, iron hematoxylin, Congo red.
- FIG. 4. Section tangential to surface of organ of Corti through first turn of cochlea. New-born kitten. Osmic acid and Zenker's fluid; iron hematoxylin and Congo red.
- FIG. 5. Section tangential to surface of organ of Corti through third turn of cochlea. Rabbit 10 hours after birth. Osmic vapors and Bouin's fluid; iron hematoxylin and Congo red.
- FIG. 6. Section tangential to surface of organ of Corti through basal part of third turn of cochlea. Dog 3 days and 10 hours after birth. Bouin's fluid, iron hematoxylin, and light green.

### PLATE 2.

- FIG. 7. Section tangential to surface of organ of Corti through middle of third turn of cochlea. Dog 12 hours after birth. Bouin's fluid, iron hematoxylin, and light green.
- FIG. 8. Section tangential to surface of organ of Corti through third turn of cochlea. Kitten 12 hours after birth. Trichloroacetic acid, iron hematoxylin, and light green.
- FIG. 9. Section tangential to surface of organ of Corti through second turn of cochlea. Kitten 3 days and 12 hours after birth. Osmic vapors and trichloroacetic acid; iron hematoxylin and Congo red.
- FIG. 10. Section tangential to surface of organ of Corti through first turn of cochlea. New-born dog. Trichloroacetic acid, iron hematoxylin, and Congo red.
- FIG. 11. Section tangential to surface of organ of Corti through third turn of cochlea. Kitten 12 days after birth. Osmic acid and trichloroacetic acid; iron hematoxylin and Congo red.

### PLATE 3.

- FIG. 12. Section tangential to surface of the organ of Corti through first turn of cochlea. New-born kitten. Osmic acid and Zenker's fluid. Iron hematoxylin and Congo red.
- FIG. 13. Section tangential to surface of organ of Corti through third turn of cochlea. Kitten 12 days after birth. Osmic acid and trichloroacetic acid; iron hematoxylin and light green.
- FIG. 14. Radial vertical section of organ of Corti through first turn of cochlea (before appearance of tunnel space). Dog 3 days and 18 hours after birth. Trichloroacetic acid, iron hematoxylin, and Congo red.
- FIG. 15. Radial vertical section of organ of Corti through third turn of cochlea (basal part). Kitten 3 days and 12 hours after birth. Osmic vapors and trichloroacetic acid; iron hematoxylin and Congo red.
- FIG. 16. Radial vertical section of organ of Corti through second turn of cochlea. Dog 3 days and 18 hours after birth. Trichloroacetic acid, iron hematoxylin and Congo red.
- FIG. 17. Section tangential to surface of organ of Corti through second turn of cochlea. Kitten 12 days after birth. Trichloroacetic acid, iron hematoxylin, and Congo red.

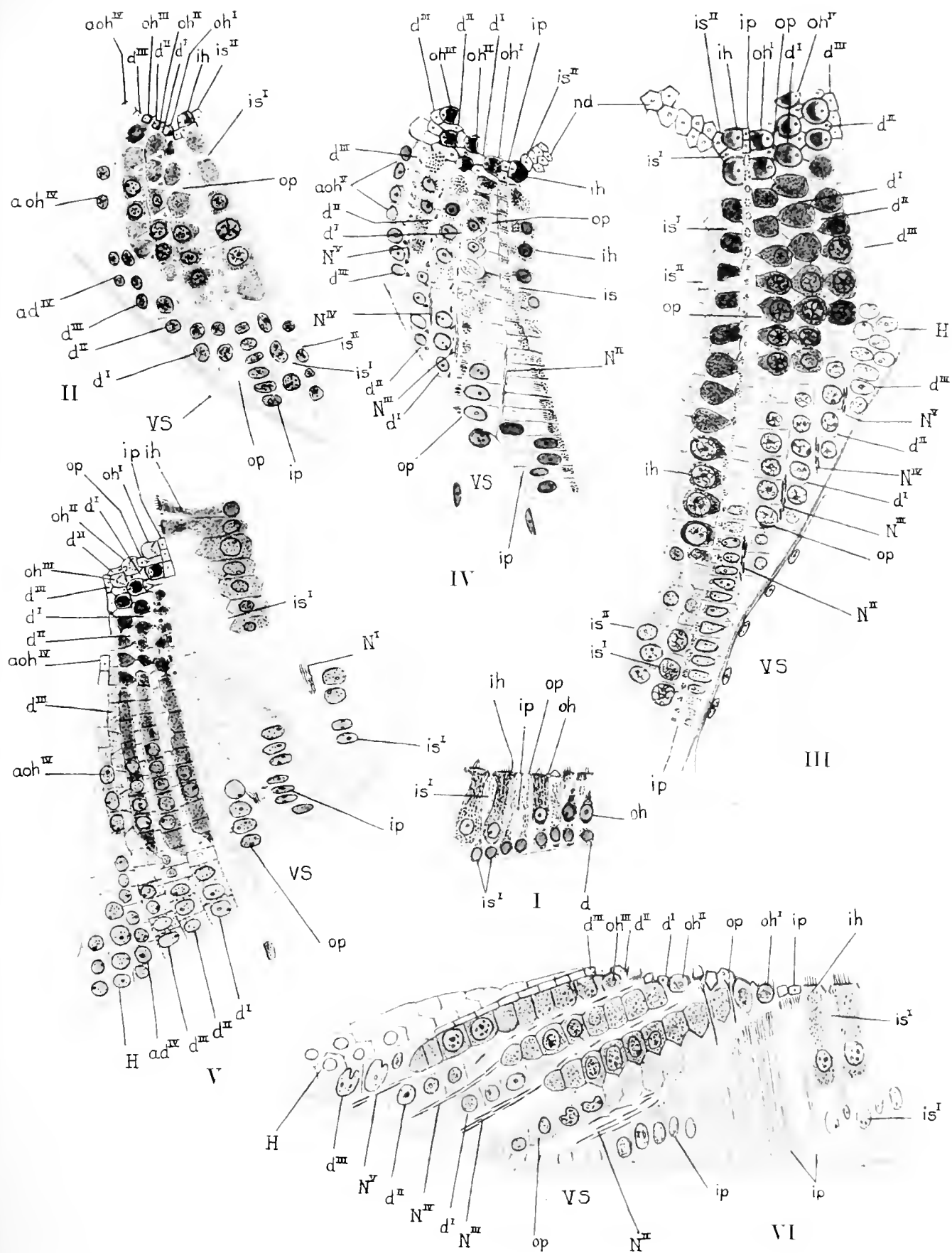
### PLATE 4.

- FIG. 18. Section tangential to surface of organ of Corti through apical part of second turn of cochlea. Kitten 16 hours after birth. Osmic acid and Bouin's fluid; iron hematoxylin and light green.
- FIGS. 19 and 19<sup>a</sup>. Vertical spiral section of organ of Corti through second turn of cochlea. New-born kitten. Osmic acid and Zenker's fluid; iron hematoxylin and Congo red.
- FIG. 20. Vertical spiral section of organ of Corti through third turn of cochlea. Rabbit 2 days after birth. Osmic acid and silver nitrate; iron hematoxylin and Congo red.
- FIG. 21. Vertical spiral section of organ of Corti through second turn of cochlea. Dog 3 days and 18 hours after birth. Zenker's fluid, iron hematoxylin, and light green.
- FIG. 22. Vertical spiral section of organ of Corti through second turn of cochlea. Dog 3 days and 10 hours after birth. Bouin's fluid, iron hematoxylin, and light green.
- FIG. 23. Vertical spiral section of organ of Corti through second turn of cochlea. Kitten 12 days after birth. Osmic acid and trichloroacetic acid; iron hematoxylin and light green.

## GENERAL ABBREVIATIONS.

adiv.....	Atrophied cells of Deiters of fourth spiral outer row.	Nii.....	Spiral nerve bundles running between inner and outer pillars.
aohiv.....	Atrophied hair cells of fourth spiral outer row.	Niii.....	Spiral nerve bundles running between outer pillars and cells of Deiters of first row.
bm.....	Membrana basilaris.	Niv.....	Spiral nerve bundles running between cells of Deiters of first and second rows.
C.....	Cells of Claudius.	Nv.....	Spiral nerve bundles running between cells of Deiters of second and third rows.
ch.....	Chromatolysis of cells of greater epithelial ridge.	nd.....	Non-differentiated cells of greater epithelial ridge.
d.....	Cells of Deiters.	oh.....	Outer hair-cells.
di, dii, diii.....	Cells of Deiters respectively of first, second, and third row.	ohi, ohii, ohiii.....	Outer hair-cells respectively of first, second and third row.
esp.....	Epithelium of sulcus spiralis.	op.....	Outer pillar-cells.
h.....	Hairs.	SN.....	Space of Nuel.
H.....	Cells of Hensen.	T.....	Tunnel space.
ih.....	Inner hair-cells.	tb.....	Terminal bars.
ip.....	Inner pillar-cells.	Vs.....	Vas spirale.
isi, isii.....	Supporting cells respectively of first and second inner row.		
N.....	Nerve bundles passing through foramina nervinum.		
Ni.....	Spiral nerve bundles running between inner sustentacular cells and inner pillars.		

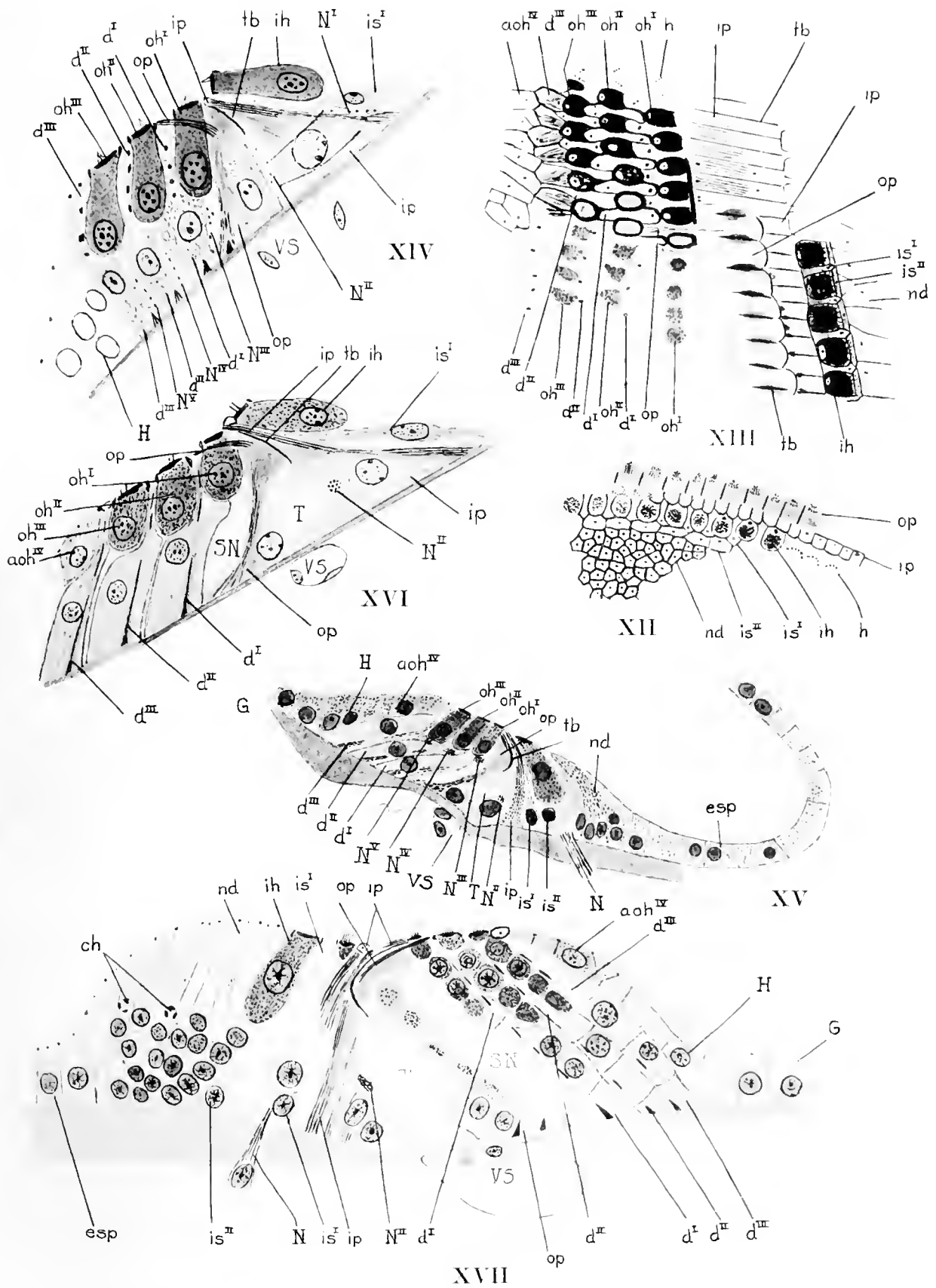




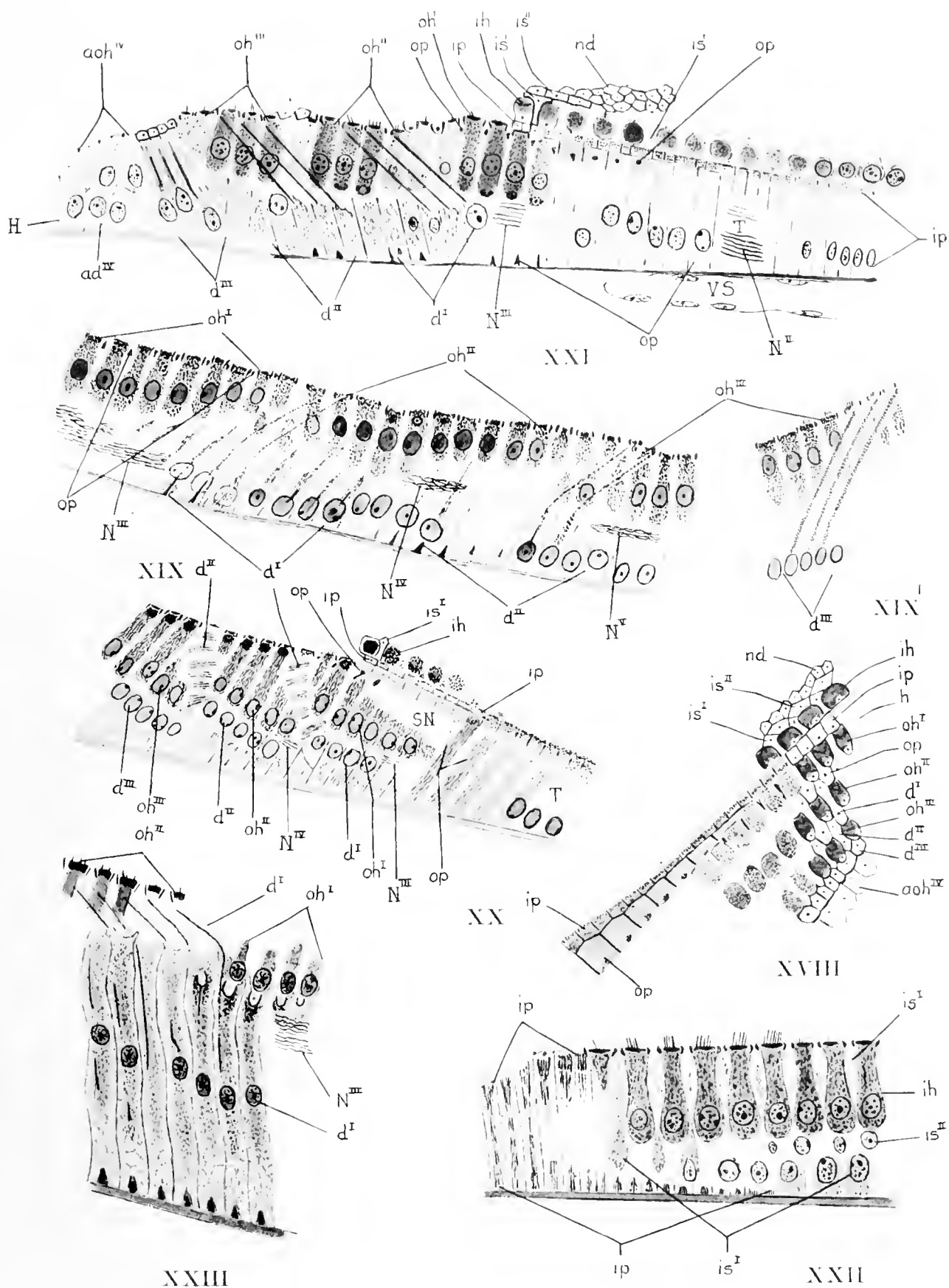
















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CONTRIBUTIONS TO EMBRYOLOGY, No. 32.

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THE SINO-VENTRICULAR BUNDLE;  
A FUNCTIONAL INTERPRETATION OF MORPHOLOGICAL FINDINGS.

BY ROBERT RETZER,  
*Professor of Anatomy in the University of Pittsburgh.*

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With one plate.

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# THE SINO-VENTRICULAR BUNDLE: A FUNCTIONAL INTERPRETATION OF MORPHOLOGICAL FINDINGS.

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By ROBERT RETZER.

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## INTRODUCTION.

In spite of the large amount of scientific work that has been done on the subject, the problem of how the contraction wave passes from the venous to the arterial end of the heart still remains unsolved. When a muscular connection between atria and ventricles was discovered and it was found that the severance of this connection brought about arrhythmia, many adherents of the myogenic theory felt that the burden of proof lay with the neurogenists. It was, however, not long before it was established that there were nerve fibers accompanying this muscular connection and the problem stood as it did before the discoveries of His and Kent. I have devoted the last fifteen years to anatomical studies bearing on this subject and during this time my views have undergone considerable change.\* In the present papers I have undertaken to outline the possible interpretations of the facts as we now know them.

It seems that there is a lack of understanding between the anatomist and the physiologist, because neither seems to appreciate the value of the facts described by the other. For instance, it should not be left out of account that the heart of *Limulus* (which is unquestionably neurogenic) has more resemblance to voluntary than to cardiac musculature, considering its morphology. Again, the peculiarity of the histologic structure of the muscular connection between the atria and ventricles and of the Purkinje fibers is left almost entirely out of consideration by those who hold that the myogenic theory is the only correct one. It is this phase of the question which is the subject of this paper.

The literature has been so thoroughly reviewed recently by others that I propose to discuss only those articles that have a direct bearing on the subject at hand. The term "atrio-ventricular bundle" was given by His, jr. (1893) to a strand of cardiac muscle which arises in the posterior wall of the right atrium, near the atrial septum, is superimposed on the superior edge of the ventricular septum with numerous interchange of fibers, and (passing forward) divides near the aorta into a right and left branch, the latter ending in the aortic leaflet of the mitral valve. It was later found that this bundle does not begin nor end in the manner described by His, but that the region of origin represents the primitive sinus region of the heart, and that it terminates in a complex network of fibers long known as "Purkinje fibers." Imbued with the idea that this connection conducts impulses from atria to ventricles, Tawara (1906) gave it the name of "Reizleitungs-

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\*Although a large part of this work was done during my association with Professor Mall, I should like also to record my indebtedness to Professor Keibel of Freiburg, and Professor Felix, of Zurich, for the courtesies extended to me while a guest in their laboratories; and to Professor Bensley for his kindly interest during my residence in Chicago.

system." This term is still used by many authors, but feeling that a morphological one must be used as long as the question of function is still in doubt, in 1908 I employed the name "sino-ventricular bundle." By this term we definitely commit ourselves only to the fact that the bundle forms an anatomical connection between the sinus region and the ventricles. The terms "conductive system" and "atrio-ventricular bundle" still have their place, as I shall indicate later.

It may be noted that most of our anatomical text-books take little cognizance of the change of our knowledge on this subject and still persist in using the term "bundle of His." It has already been pointed out that His's description is far from correct. I may add here that considerable skepticism as to the correctness of the observations of His existed in the mind of his father and of other mature anatomists when I made my first observations under Professor Spaltholz, in 1903, and it was for this reason that the problem was undertaken again. Kent's (1893) original description is far more correct in the light of our modern investigations than was that of His, and the bundle has been named the "bundle of Kent" by some authors; but we should recognize the fact that it was His, who by experimental methods, was the first to attempt to prove that all impulses from atria to ventricles pass by way of the bundle.

In my notes, made in 1903, when I began my studies on the subject, I find the following review of Kent's article:

"In his figure on p. 244, it seems to me that he mistakes the Purkinje fibers for fibers of the myocardium. His description of the cells standing between muscle and connective tissue reminds me also of these (Purkinje fibers)."

This note was made five years before the appearance of Tawara's monograph, which showed indisputably that the Purkinje fibers represented the end-ramification of the atrio-ventricular bundle. Recently I read the Proceedings of the Physiological Society of November 12, 1892, which I had previously overlooked, and I was much interested in the report of Kent's paper before the society:

"Between the auricle and ventricle and lying in the connective-tissue ring are modified muscle cells, usually spindle-shaped, nucleated, granular, becoming extremely narrow in parts and then swelling out again, transversely striated, branched, and usually connected into a network. These cells are somewhat rudimentary in the case of the rat and are very well developed in the monkey. In the latter animal they exist as a complete network, permeating the fibrous connective tissue of the groove and extending through from auricle to ventricle. Upon approaching the groove the normal cardiac fibers split up into similar fibers and become connected with the network of cells lying in the fibrous tissue."

This shows even more conclusively that Kent did recognize the difference in appearance between the bundle-fibers and the heart-muscle fibers and also saw the ventricular transitions between them, although he did not recognize that the end-ramifications of the bundle really represent the Purkinje fibers. His description, however, lacked the dogmatism which seems so essential for the general acceptance of a new idea. Thus he states in a summary:

"It would *appear*,\* then, that the fact of two masses of muscle being joined together by fibrous tissue is in itself no argument against the muscular continuity of such masses,

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\*The italics are mine (R. R.)

the fibrous nature of the intervening tissue by no means excluding the possibility of muscular fibers running through it and preserving the muscular connection. And in the mammalian heart such a connection *appears* to exist."

Kent (1913) finds more than one connection in some hearts he examined and all careful researches will confirm this observation. In the pig I have seen bundles of muscle-tissue streaming down along the outer posterior wall connecting the muscular tissue in the region of the coronary sinus with the ventricular musculature.

Since then innumerable papers on the subject have appeared and some authors have claimed priority in the discovery of the "bundle," either for themselves or for others. Thus priority was claimed by Paladino (1914) and some authors contend that Kürschner described the bundle in Wagner's Handbuch. These authors did describe muscular connections, but unlike His, they did not limit these to a restricted area of the septum. Until the monograph of Tawara appeared all work done was in the main confirmatory of His. According to those authors, the muscular connection consisted of ordinary cardiac musculature and contained but a negligible amount of nerve-fibers. Tawara was the first to show the connection between the bundle and the Purkinje fibers, although (as I have stated above) Kent had appreciated that there was a difference in the histological structure of the bundle and the rest of the cardiac musculature.\*

Tawara called this connection between atria and ventricles and the widely ramifying Purkinje fibers the "Reizleitungssystem." He described nerve-fibers accompanying the system but paid little attention to them. Indeed, his monograph was used extensively by the adherents of the myogenic theory as further evidence in their favor. In spite of the careful work of Erlanger and others, it has never been determined definitely whether it is the nerve-fibers accompanying the bundle or the bundle itself that is the conducting element. The term "conductive system" is, therefore, only applicable to the nerve-muscle complex. It must be divided anatomically and physiologically into its component parts—the nervous mechanism and the muscular mechanism. This is essentially the problem of to-day. Its solution will definitely settle the myogenic or neurogenic theory of the mammalian heart-beat.

Authors using the term "bundle of His" usually mean the muscular connection. They use the term "Tawara's node" also in the sense that it is a muscular node—as described by Tawara. In view of the fact that the bundle is in all instances accompanied by nerve-fibers, we must be more accurate in our terminology. Therefore, it has been the author's custom to use the term "conductive system" not in Tawara's sense, but as a designation for the *neuro-muscular* complex extending from an indefinite area in the wall of the right atrium to the ventricles including the Purkinje fibers. His's bundle or "atrio-ventricular bundle" I have termed the *muscular* connection between atria and ventricles, not including the Purkinje fibers. The term "sino-ventricular bundle" is used to define the *muscular* con-

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\*Although Tawara's work has been confirmed in this respect by all who have published on the subject since then, we find in the 1912 edition of Quain's Anatomy, Schaefer's Text-book of Microscopic Anatomy, the following statements, p. 200: "In man and most animals, distinct Purkinje fibers do not exist, but the cardiac fibers are rather larger near the ventricular endocardium than elsewhere." And further on: "In animals which have fibers of Purkinje, the ventricular end of the bundle resembles these in structure." Statements like these in text-books which we have learned to look upon as more or less authoritative should be corrected.

nection extending from the region of the coronary sinus and the great veins to the ventricles, including the Purkinje fibers. The nervous mechanism is without a name.

#### ANATOMICAL DESCRIPTION.

The general gross anatomy of the muscular connections has been so ably described and so well illustrated by Aagaard and Hall (1912) that it would seem unnecessary to describe it again were it not that slight differences of opinion concerning the extent of these connections necessitate a definite statement upon which I base my later discussion. We generally find the descriptions and figures begin with the node of Tawara and end with the ramification of the Purkinje fibers. Such descriptions are quite correct as far as one can recognize these structures in the gross. Injection methods which give most satisfactory results for demonstrating the system are useless beyond these areas, and it is doubtful whether any other gross method will reveal anything further that can be considered conclusive. The use of the Zeiss-Greenough binocular in dissecting out the sinus portion will show fibers leading from the node to the coronary sinus and inferior vena cava, but whether these fibers are continuous with the bundle or not is a question that needs close histological examination. Both with the gross and with the microscopic investigation there seems no difficulty, as long as the bundle is invested by fibrous sheath. It is this fibrous sheath which is injected, and it is this sheath also which stands out most prominently when stained with dyes that differentiate between musculature and fibrous tissue. As soon as we reach the node of Tawara, when tracing the bundle to its origin, the fibrous sheath disappears, and from here on the tracing is difficult, if not impossible, by any gross methods. Similarly, at the other end of the bundle, we can not demonstrate in the gross the transition between it and the cardiac musculature.

The description of the gross, therefore, will closely correspond to the description given by most writers of recent years, but to make it complete we must add to it what the microscope reveals. The bundle takes its origin in the musculature of the right side of the interatrial septum immediately in front of the coronary sinus or of the left superior vena cava in those mammals that lack a coronary sinus—that is, in those that have a persistent left superior vena cava. It passes forward and downward\* in the septum and comes to lie beneath the insertion of the septal leaflet of the tricuspid valve. This part of the sino-ventricular bundle is so delicate and diffuse that it can not always be distinguished from the surrounding atrial musculature, even after the removal of the endocardium. Here the diffuse strands are collected into a bundle which can readily be seen as a light-colored strand having a diameter of 2 to 3 mm., running perpendicularly to the direction of the fibers of the ventricular septum and between them and the pars membranacea septi. At a point that lies in a line drawn from the apex of the left ventricle to the junction of the right and of the posterior cusps of the aortic valve, it divides into a right and a left branch that straddle the muscular interventricular septum. These two main branches pass slightly forward and downward toward

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\*The directions refer to the excised heart with base upward and septum antero-posterior.

the base of the papillary muscles. In the left ventricle, there being two papillary muscles, the bundle must necessarily divide, while in the right ventricle it remains single, reaching the large papillary muscle by way of the moderator band if there is one, or in relatively the same position if it is lacking. In the left ventricle the main branches are visible without dissection, appearing as light strands underneath the endocardium, sweeping downwards towards the base of the papillary muscles making an inverted V. In the right ventricle the bundle is frequently beneath a millimeter or more of musculature, but becomes visible at the base of the papillary muscle. The end-ramifications or the Purkinje fibers are quite visible in the fresh heart, but in many of the preserved hearts they can not be defined. They spread out underneath the endocardium in a complex network, usually bridging over the recesses between the trabeculæ carneæ.

This description is applicable to all mammalian hearts. In the human heart as well as in the dog's, it should be noted that the strands are exceedingly delicate and tear easily when dissected out. In the Herbivora, however, the entire system is ensheathed by collagenic fibers that give the bundle a very much lighter color than the surrounding musculature and also make dissection easy. In these species, sheep especially, the Purkinje fibers are accompanied by fat-cells that stand out gray on the dark background. In these hearts, furthermore, the right branch lies immediately beneath the endocardium, while in the human and dog it must be dissected out of a depth of a millimeter or more of musculature.

The microscopic anatomy is far more complex than the gross. It is due to inadequate histological work that our present conception of the nature and function of the sino-ventricular bundle is somewhat erroneous. The microscope must settle two questions—one, the origin of the bundle where we lose trace of it by dissection, and the other, the nature of the material that constitutes the bundle. In reference to the first question I must confess that we have not advanced far. As previously stated, it is my opinion that the bundle does not originate in Tawara's node but in the musculature that represents the primitive sinus region of the heart. I base this opinion upon the examination of the embryonic heart, where the musculature of the various parts presents greater cytological differences than it does in the adult. In the latter the problem is similar to tracing finer nerve tracts in the medulla without the use of pathological material which singles out these tracts by degeneration. Nerves, connective tissues of various kinds, and poorly striated musculature interwoven in a most complex manner present almost insurmountable difficulties. In reference to the second question we realize that our histological knowledge is based almost entirely upon Tawara's work. His illustrations are more or less diagrammatic and for the sake of clearness such structures as seemed to him unessential were omitted. He showed that there were histological differences between various mammals and in various parts of the same heart. But as to why we have these differences he does not even speculate. I think that we can show the reason of these differences and what their significance is.

As that material was at one time most available, I used the pig heart for study. For comparison, many other hearts were studied, including the human. Various methods were used, such as the maceration, the injection, the dissection, and the use of other reagents to bring about a clear picture of the system in gross, but, as stated before, it meant merely a repetition of what is already known. I was, therefore, forced to depend entirely upon histological technique. At first I was content with the use of such fixatives as Zenker's, formaldehyde, mercuric bichloride, and other combinations, and with such stains as would differentiate between muscular tissue and connective tissue. It was soon seen that these methods would not lead me beyond the confirmation of previous work. As stated before, as long as we have an investment of the bundle there is no difficulty in tracing it, but I can state from broad experience that no one who is not thoroughly conversant with the finer structures of cardiac musculature can distinguish between a few cells of the bundle and those of ordinary cardiac musculature. This is especially true of the human heart.

With Zenker's fixation and hematoxylin and eosin stain, or hematoxylin and van Gieson, there is one characteristic common to the entire system, and that is a clear perinuclear space. The nature of this perinuclear space will be discussed later.

Let us look more closely into the histology and cytology of the conductive system in the pig's heart. For descriptive purpose we divide the system into five parts: the atrial part, the junctional part, the ventricular stems, the preterminal part, and the terminal part or Purkinje fibers. There is a gradual transition from one part to the other, but the difference between the first and last is very great.

The first part begins in the region of the great veins and ends in the node of Tawara. It is most difficult to tell where this musculature begins and where the atrial musculature ends, but if one looks underneath the endocardium or underneath the intima of the great veins one will see the clearly defined cells with a perinuclear space. The fibrils are not as numerous nor is the striation of them as marked as in the atrial musculature.

It is a matter of opinion whether these cells around the great veins belong to the sino-ventricular bundle or to the system belonging to the Keith-Flack node. It makes but little difference, because one system is connected with the other and it becomes more a question as to where one ends and the other begins. It is my opinion that we can not make any anatomical distinction between the Keith-Flack system on the one hand and the sino-ventricular on the other. Physiologists may find an easier explanation of cardiac phenomena by considering these systems separate and distinct, but we have no anatomical proof that such a condition exists.

The second or junctional part is what is known as Tawara's node. In most animals this node is situated in a restricted area between the trigonum fibrosum and the endocardium in the right side of the interatrial septum immediately above the interventricular septum. In most mammals (including man) the trigone above mentioned contains either bone or cartilage. It is owing to this fact that thin coronal sections of this region are most difficult to obtain. The necessity of



using an acid fixative in order to decalcify and soften the tissue prohibits the use of finer cytologic stains. It is therefore very fortunate that in the pig this node is not restricted to this limited area but streams down into the ventricles, accompanying and gradually merging into the third part.

In figure 1 this area is shown. The section is taken parallel to the ventricular septum and lies a little below the atrial junction. At *a* is shown the ventricular musculature with many nuclei in the same fiber; between *a* and *b* is loose connective tissue which forms Curran's (1910) bursa and which can readily be injected; *b* shows the third part and *c* the second part. It will be noted that it consists of an intricate interlacement of fibrils that show but little cross striation. These fibrils constitute a syncytium that incloses clear areas containing nuclei. It will be noted that there are generally two nuclei in the same area. In several places, where but one nucleus is drawn, another one can be found in a lower level of the section. Again the membrane of one nucleus is directly continuous with that of another. Unquestionably we are dealing here with amitotic nuclear division.

The transition from the second to the third part of the system is greater than between any other two parts. The third part or ventricular stem (*b*) shows an enormous increase in the number of fibrils. It is difficult to determine whether this increase has been brought about by a splitting of the fibrils or whether it has developed from a differentiation of the cytoplasm. Each nucleus is now surrounded by its own perinuclear space. The bundle is here divided up into a number of strands by the invasion of delicate collagenic fibers and presumably nerve-fibers.

It seems that we are dealing here also with a syncytium, as far as the fibrils are concerned. Nevertheless, there is a definite condensation of fibrils midway between the nuclei. This gives it an appearance that would suggest cellular delimitations. The question whether the fibrils would then be considered as exoplasm or as extracellular deposits lies without the scope of this paper.

In the fourth or preterminal part (see fig. 2) we find the cell area increased in size and two nuclei are found to be situated in the same perinuclear space, which again indicates a multiplication of nuclei, as it does in the second part. The fibrils are less numerous in a given area than they are in the second part of the bundle. We find a considerable increase in the amount of collagenic fibers, which we recognize in the gross as the sheath of the bundle.

In the fifth or terminal part (see fig. 3), which constitutes the Purkinje fibers proper, we find that the nuclei have moved to the border of the cell area. Each one is surrounded by its own perinuclear space. In the pig we find as a rule four nuclei for one cell area. The striation of the fibrils is a little more marked than it is in the other parts of the bundle, but not as much as in cardiac muscle proper. The collagenic fibers are restricted to the border. A few delicate strands can be seen which delimit the cell areas. In various parts (shown at *d*) of this Purkinje system we see transition to the cardiac musculature; this transition consists in an increase and a more longitudinal arrangement of the fibrils and a further multiplication of the number of nuclei. In the pig this nuclear multiplication brings about rows of nuclei, as many as eight, that are situated in the central core of the fiber.

The description just given applies only to the pig's heart. Similar conditions will be found in other Ungulata, but in the heart of man and of Carnivora one would have difficulty in recognizing the cellular multiplications just described. The first thing we have to consider is that in the Carnivora there are but two nuclei to a heart-muscle cell, while in the pig there are eight. In the production of an adult human cardiac muscle-fiber we would see but one-fourth as many nuclear divisions as we do in the pig. These nuclear divisions seem to be restricted to the first and second part of the bundle and to the Purkinje fibers. The appearance in the human is, therefore, not as striking as it is in the Herbivora.

The cytologic details of the various parts of the bundle do not differ as markedly as one might expect from the low-power examination. As in cardiac muscle, we must distinguish three parts of the cell: the fibrils, the sarcoplasm around the fibrils or sarcostyle, and the perinuclear sarcoplasms.

It has been previously pointed out that it is not difficult to trace the bundle from Tawara's node to the Purkinje fibers in sections that have been stained with hematoxylin and van Gieson stain, or with Mallory's connective-tissue stain, because these stains bring out the fibrous investment of the bundle very clearly. One of these stains has been used by every pathologist who has reported on the histological findings of Stokes-Adams disease. Many will admit that they would be absolutely at a loss if they were to use a general stain, such as hematoxylin and eosin. Further, it is evident that thick sections studied with the low powers of the microscope make tracing of the bundle easier than thin sections with oil immersion. The reason lies in the fact that the differences, in the human heart especially, between the bundle cells and normal cardiac muscle cells are so slight that they become evident only when seen en masse. The optical difference is primarily due to a proportionately larger amount of sarcoplasm in the bundle fibers than there is in the musculature. Sarcoplasm does not stain as heavily as do fibrils, with the result that the bundle always stands out in thick sections as a light-colored area. It is obvious that the thinner the section the more do these color differences disappear.

I have endeavored to analyze the chemical nature and the cytologic details of the sarcoplasm because I felt that an analysis of this cell element would give us a clue as to the nature of the bundle. I attempted to determine the glycogen, lipid, and protein content and the mitochondria. I regret to say that I was only partly successful. As regards the glycogen content, we confirm the work of Aschoff (1908), who finds but very small amounts in the pig's heart. It should be noted, however, that in the heart of beef and sheep the glycogen content far exceeds that of the ventricular musculature. Indeed, it is not difficult to trace the course of the bundle by means of the glycogen reaction. The reason the pig's heart has so little is due to the fact that the nodal tissue which is restricted to the second part of the bundle extends far into the ventricles. The sarcoplasm is much reduced. The results in the heart of the rat were negative, but as only two hearts were examined I do not consider them conclusive. The preponderance of evidence (see results and discussions by Engel, 1910, and Aschoff, 1908) lies in favor of the view that

the bundle-fibers contain a larger amount of glycogen than do cardiac fibers. But the ratio seems to be in the same proportion as the amount of sarcoplasm.

A study of the lipoid, protein, and mitochondria content can not be dissociated. Mitochondria or chondriosomes are morphological constituents of the cell, of whose chemical nature we know but little. The lipoid elements may appear in the form of granules, liposomes, or may be attached to or a part of the mitochondria. As to the protein, myogen, and myosin, we may say that it can be either in soluble form and optically inactive or attached to a part of some of the morphological constituents which have been lumped under the term *interstitial granules*. We must make a distinction, however, between the granules which are situated between the fibrils or sarcostyles and those that are found in the perinuclear space. My work has led me to a closer analysis of these structures, but I can not say that the results are conclusive. I therefore make the following statements with some reservation:

Some interfibrillar granules seem to bear the same relation to the fibrils, in regard to both size and structure, in the bundle fibers as they do in cardiac muscle. I am inclined to consider them as contraction phases in Holmgren's (1910) sense. There are others which have been called mitochondria, but as they do not give all the mitochondrial reactions I am doubtful of their nature. Mironesco (1898) has found, however, that a large amount of mitochondria are present in the Purkinje fibers; he concludes that the latter represent a reserve material for cardiac musculature. Duesberg (1910) has shown (and so far his work has not been disproved) that myofibrils arise from mitochondria or chondriosomes. The perinuclear granules, however, are both larger and more numerous in the bundle, especially in the terminal portion, than they are in cardiac muscle. Some of these granules give the glycogen reaction, while others show that they must be lipoid in nature.

#### FUNCTIONAL INTERPRETATION.

The object of this paper is to give a physiological interpretation of the facts that are brought forth by careful anatomical observations. We realize that to a great extent the study of function belongs to the physiological laboratory; but when we realize that in former years the physiologists were also the histologists, and that furthermore much of our early physiological knowledge was derived purely from microscopical studies, it may not be amiss to again use our anatomical knowledge for the explanation of physiological phenomena. In doing so we fully realize that it is speculative, but it has its justification if it can prove of value in directing the efforts of the experimentalist.

McCallum (1898) stated that the ultimate number of muscle-fibers in voluntary muscles is laid down at birth. Increase in the amount of musculature after this time is due to the increase in the number of fibrils. The same was held to be true for the muscles of the heart. It seems inconceivable to me that muscle-cells should differ fundamentally from all other cells which have even a less constant activity. We know that gland cells are replaced. Why should not muscle-fibers be replaced? This question seems especially pertinent in reference to the heart.

Can this organ, which has a continuous activity, contract every minute of the day and night from several months before birth until maturity and death, without its constituent elements being replaced or rejuvenated? We find nothing in the protoplasm of musculature that seems fundamentally different from the protoplasm of any other cells, nor does chemistry reveal any reason why muscle-cells should live far beyond the life period of other cells. It is true that we have not observed the constant degeneration and regeneration that is so evident in many other tissues, but I take the view that they are nevertheless there and can be seen and their presence demonstrated if proper cytologic methods can be found.

In many organs of lesser activity we have found evidence of such replacement, and no one to-day can fail to see them in epithelial structures. In these cases we have been led to the interpretation of cell-replacement by the fact that we frequently recognize that one type resembles closely the cells that predominate in the embryo. In certain instances we may see distinct evidence of the degeneration of the superficial cell layer or of individual cells, and then again these may become evident only in pathological conditions. In the heart we likewise have two or more types of cells, one of which, the cells of the atrioventricular bundle, resembles the embryonic heart-muscle cell. To argue that Purkinje fibers are already differentiated in the embryo and do not present in the adult morphological features identical with those found in the embryonic state is puerile. We have no difficulty in recognizing as distinctive the various layers of embryonic squamous epithelium, and no one would mistake them for adult; yet no one doubts that the superficial layer in the adult is continually being replaced by the cells beneath—which, though not embryonic in appearance, yet divide and multiply as the embryonic cells. The same structures which in the embryo grow and multiply by a process of karyokinesis, in the adult absolutely fail to show such evidences of multiplication. Similarly, as Bensley (1911) has shown in the case of the pancreas, the duct-cells represent the multipotential elements, which are embryonic in every sense but their appearance, yet nevertheless produce both islet and acinus cells. In other words, if we argue that Purkinje fibers can not be embryonic in type because they are morphologically unlike the embryonic cells, we must likewise concede that because the deeper layers of stratified epithelium are not embryonic in appearance they can not replace the superficial, and that duct-cells can not replace islet cells and acinus cells because they are unlike them.

As regards the degeneration process, I must state that I have no evidence to present. I have looked for proof of my assertion, but the same difficulty presents itself that is found in studying the origin of myofibrils. After employing methods such as are used for fixing and staining mitochondria, I find in the embryonic musculature rods that have a definite color reaction. Suddenly we have fibrils that have a slightly different tinge. If these fibrils are derived from these rods a certain chemical or physical change must have taken place that brings about a change in color reaction. Likewise we would expect a similar change of reaction when the myofibrils break down. A single degenerated muscle-fiber is difficult to distinguish. We know that in certain types of degeneration the first sign of degeneracy is the

disappearance of isotropic and anisotropic bands and a reduction in the amount of sarcoplasm. The nuclei appear shrunken. The appearance is not at all unlike a strand of collagenic tissue and undoubtedly many such degenerated muscle-cells have been mistaken for connective tissue.

It is therefore our opinion that the sino-ventricular bundle is to cardiac muscle what the deep layer is to the superficial in transitional epithelium. It represents the center of growth of heart muscle. It grows from the sinus region, the region which is the most primitive of the heart, and by a series of cell divisions reaches the ventricles of the heart to replace the worn-out heart-muscle cells. That is its essential function. When we study the development of the heart (cf. Mall, 1915) we readily see that the course of the bundle is predetermined by developmental changes taking place in the primitive heart tube. In other words, the bundle takes the shortest course possible between the sinus region and the ventricles of the heart.

It has been pointed out that nerve fibers always accompany the bundle, and thus far it has not been determined whether this accompaniment is due to physiological association or is merely incidental. In the early embryos ganglion cells are distinguishable in the atrial septum in the immediate vicinity of the bundle, and it is but natural that their processes should take the same course as do the bundle-fibers, because during development nerves always take the shortest course to their sites of innervation. It is, however, significant that Wilson (1909), Morrison (1912), and others have shown nerve terminations on the bundle. Basing it upon their observations, there are some who are therefore inclined to argue that the bundle is a specialized neuromuscular mechanism; but as the nature of these terminations is not known and as there are likewise similar nerve terminations to be found in other parts of the heart, it seems unwarranted to assume that there exists a physiological difference between them. It is our opinion that these nerve terminations have their origin in postganglionic neurones and most likely form a synapse with the vagus terminations directly or through the intervention of another neurone.

Most experimentalists agree that after section of the conductive system stimulation of the vagus will not inhibit the ventricular beat. This bears out the anatomical observation, namely, that the bundle is sinus tissue and naturally in its growth down to the ventricles brings with it the nerve that is associated with it—viz., the vagus. I do not mean to imply that in these experiments vagus fibers were necessarily cut. If the conduction passes by way of the sino-ventricular bundle the very origin of the latter will explain the reason of the lack of response to vagus stimulation upon the section of the area in question. The accelerator fibers follow the course of the coronary vessels and are not anatomically associated with the bundle.

The work of Burrows (1912) dispels any doubt that may have existed that embryonic mammalian heart-muscle has the power of rhythmic contraction. Embryonic tissues have, however, greater potentiality than adult structures and it is quite conceivable that in the adult the muscle-tissue has become so specialized that it retains its power of contraction but has lost its power of conduction. If this is the case, conduction takes place by nervous pathways, but it should be remembered

that the sino-ventricular bundle is not adult muscle. It should be noted in this connection that as long as there is a complete muscular continuity between the atria and ventricles in the embryo, ganglion cells are not visible in the heart; but ganglion cells appear with the invasion of the atrioventricular ring by connective tissue and the formation of the valves. In other words, before the definitive physiologic reactions take place there are important morphologic changes, namely, the appearance of cross-striations, of ganglion cells, and of nerves.

Attention should again be called to the accessory muscular connections that are found between the right atrium and ventricles. No experimentalist should leave these out of consideration when interpreting the results of his findings. It belongs to future anatomical researches to establish whether these connections also have their origin in the sinus region of the heart or whether they are truly atrioventricular fibers.

To sum up, therefore, we have in the sino-ventricular bundle primarily a growth center for the replacement of cardiac musculature. The cells begin their growth in the sinus region of the heart and by a series of direct nuclear division eventually reach the subendocardial musculature where they pass into cardiac-muscle cells. Being embryonic in type they may retain their embryonic bipotentiality of contractility and conductivity. The nerves which accompany the bundle are associated with the vagus nerve as is the sinus tissue which it innervates.

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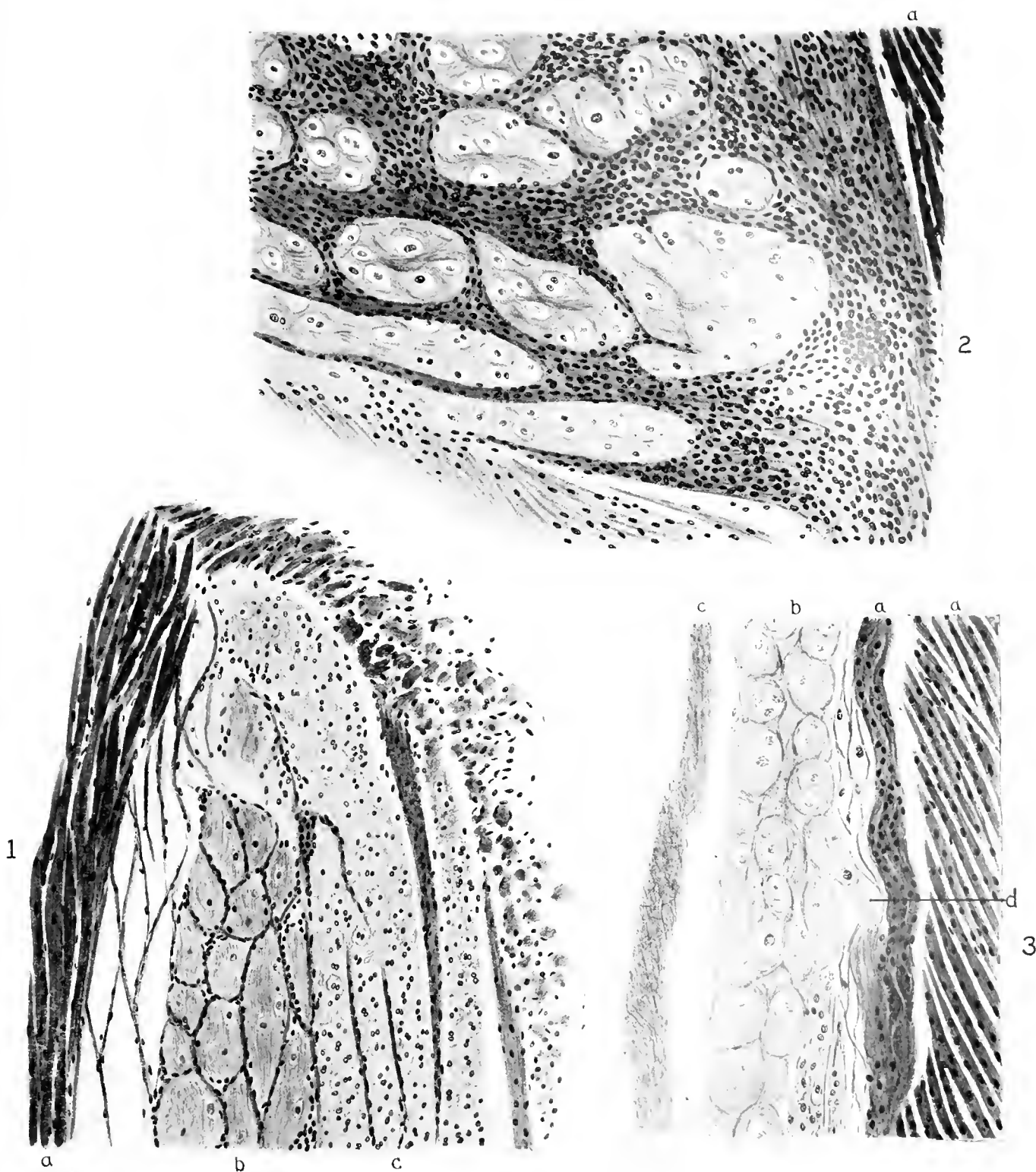


FIG. 1. This shows the second (c) and third (b) parts of the sinoventricular bundle invading the ventricular musculature (a). The upper part of the figure points toward the apex of the heart. Between the cardiac muscle (a) and the bundle (b, c) is seen Curran's sheath.

FIG. 2. The preterminal part of the bundle is here shown, lying in a dense mass of fibrous tissue. The size of the cells should be compared with the ventricular musculature.

FIG. 3. This illustrates the terminal part of the sino-ventricular bundle or the Purkinje fibers (b) and a transition to the cardiac muscle (a) shown at (d). C is endocardium.

The drawings were all made from the same series. The sections are from an adult pig's septum fixed with Telly-sniczky's fluid and stained with hematoxylin and eosin. Enlargement 210 X.





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CONTRIBUTIONS TO EMBRYOLOGY, No. 33.

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A STUDY OF THE SUPERIOR OLIVE.

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With two plates and one text-figure.



# A STUDY OF THE SUPERIOR OLIVE.

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BY GEORGE B. JENKINS.

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Some years ago, while working under the stimulating influence of Dr. Mall, I undertook at his suggestion the study of the morphology of the human inferior olive. During the progress of that work I determined to extend the investigation to include a detailed study of all the cell collections in the brain stem, both in man and in such of the lower animals as might be available. I now wish to present some of the results of this study as applying to the so-called *superior* olive, the literature upon which subject is so contradictory and confusing that one gets but a vague idea of this nuclear mass.

It seems probable, from a study of the earlier literature, that the term *olive* was applied to the inferior or bulbar olive because of the presence of the oval or olive-shaped prominence upon the exterior of the ventro-lateral surface of the medulla; and that later, when the underlying nucleus was discovered, it was quite naturally termed the *olivary nucleus*. The only apparent reason for calling the smaller pontine nucleus the *superior olive* was the assumption that it was related to and of similar cell content as the earlier-discovered inferior body. Neither of these conclusions has been borne out by my findings.

In order to arrive at a clearer comprehension of the superior olive, it was determined to use cat tissue for a preliminary study, since all investigators seem to agree that this animal has a large and beautifully developed nucleus. A number of other animals, including dogs, rabbits, field-mice, rats, and ground-squirrels, were used as checks, and all findings were compared with human tissue of varying ages. As a result of these studies the more representative types were found to be the cat, dog, and man. The tissue figured herein consists of the brain-stem of an adult cat, serial No. Fel. 1, cut in transverse sections 20 microns thick and stained with hematoxylin and eosin; the brain of a dog-fetus, 115 mm., serial No. Can. 1, cut in transverse sections 20 microns thick and stained in Ehrlich's hematoxylin; the brain-stem of an adult dog, serial No. Can. 4, cut in transverse sections 20 microns thick and stained in hematoxylin and eosin; the brain-stem of a human fetus 168 mm. (CR), serial No. Hu. 28, cut in transverse sections 20 microns thick and stained with borax carmine and Lyons blue. All were reconstructed by the Born method, using 2-mm. wax plates. All of the sections of the human specimen were drawn at a magnification of 100 diameters; in the dog and cat specimens every alternate section was drawn at 50 diameters. The adult dog was not modeled, since the outlines of the nuclear masses in the two animals are identical in all essential particulars; and, owing to the comparative scarcity of associated fibers and the simplicity of the cell components, the younger animal offered fewer mechanical

difficulties in the way of modeling. The adult was used as a control and for fiber and cell study. In each case one-half of the section was drawn and the median line was used as a straight edge to pile by, a wood form being used as a guide, as described in the study of the inferior olive. It was thus possible to pile by the external form of the pons and the ventricular floor, the perpendicular being the fixed plane.

The primary requisite, as shown above, was the selection of such ages and animal forms as would most accurately depict the typical external form of the nucleus. A second condition necessary was the selection of tissue of such a stage of development as would show the fully developed cells in a given animal; *e. g.*, while the fetal dog presented a gross nuclear form and arrangement practically identical with that of the adult animal, the fiber arrangement and cell make-up were entirely different, the younger animal presenting the embryonic cell type, the adult showing the spindle cell which was found to be characteristic of this nucleus.

The superior olive is found in the lateral field of the pons, in the ventral portion of the roughly triangular interval between the nervus abducens and the emergent portion of the nervus facialis, and lies in an indentation in the dorsal surface of the corpus trapezoideum, ventro-mesial to the nucleus nervi facialis, and occupying the lower half of the vertical extent of the pons. The mass begins caudally at the lower limit of the pons, the exact level varying somewhat in different subjects or even slightly on the two sides of the same subject, and extends cerebrally well up into the region of the nervus trigeminus, the upper limit likewise varying. It is placed definitely dorsal to the trapezium, the fibers of which curve ventrally around the nucleus, few, if any, passing dorsal to it. The nucleus nervi facialis, beginning caudally in the upper medulla, extends well up beyond the middle of the olive and lies almost in contact with its dorso-lateral surface. Where not in relation to the trapezium and the nucleus facialis, the superior olive is surrounded by the *formatio reticularis* of the pons. The nucleus nervi abducentis appears subjacent to the ventricular ependyma, within the loop of the nervus facialis, and at the vertical mid-level of the olive.

Certain of these general figures vary considerably in the different animal forms, in keeping with the laws of development. We find, for example, in a comparative study of this area, abundant evidence that the degree of development of the pons is directly proportionate to that of the cerebellum; and further, that the relations of the various structural and contained elements vary in accordance with the degree of development of given parts. The human subject, with its well-developed cerebellum, presents large numbers of cortico-rhombic and cortico-spinal efferents (pyramidal fibers), and especially large numbers of transverse pontine fibers, both superficial and deep to the cortical efferents; also proportionately large numbers of cells (the *nuclei pontis*) packed within the interspaces between these fibers. As a natural consequence, the superior olive, in this type, is much more deeply placed and farther from the ventral periphery of the pons; whereas in the lower animals, such as the dog and cat, in which the cerebellum is not so well developed, we find small pyramidal bundles lying entirely superficial to much less numerous transverse pontine fibers, with comparatively few and scattered cells representing the *nuclei*

pontis. Hence in these types the olive is much nearer the ventral surface of the pons. It was found to be more superficially placed in the cat than in the dog.

This area of the pons is very vascular, comparatively large vessels cutting into and between the portions of the nuclear complex. In the medulla the vagus and hypoglossal nerves embrace the inferior olive, the latter nerve cutting the nucleus and partially separating the so-called accessory from the main nucleus. In the pons we find the same arrangement to some degree, with the abducent and facial-nerves embracing the superior olive. In the cat and man, however, the abducens runs well medial to the superior olive; whereas in the dog it comes in contact with the medial margin of the nucleus, even cutting through it in one specimen examined (fig. 5). In man the emerging facial fibers run closer to the olive than do the abducent.

Functionally, the superior olive is to be classed with the cell masses which are developed in relation to the special sense organs. That this nucleus is a way-station in the auditory pathway is borne out by a study of its fiber relations, which will be discussed later on, and by pathological conditions produced experimentally. Baginsky claims that destruction of the cochlea in a new-born animal is followed by atrophy and disappearance of the superior olive of the same side; and von Monakow found that sectioning of the lateral lemniscus in one of the lower animals (cat or dog) was followed by atrophy and disappearance of the dorsal portion of the superior olive of the same side. He assumes from this that only a portion of the cells of this nucleus stands in relation to the lateral lemniscus. Other studies have established the fact that fibers, axons of the cells of the ventral cochlear nucleus, run transversely across the pons, forming the corpus trapezoideum, and that a portion of them, at least, terminate among the cells in the superior olivary nucleus. Flechsig suggested that the superior olivary nucleus might be concerned with the innervation of the muscles of the ear, as it is considerably larger in animals with large, very movable ears. This contention is successfully disposed of by Spitzka, who states that he has found this nucleus highly developed in the cetaceans.

The superior olivary nuclei in all of the animals studied present a sufficient degree of similarity in gross morphology, histologic constituents, and relations to enable us to establish a definite type of nuclear mass as a standard which can be used as a basis for further study and comparison. The conformity to type presented by this nucleus in each of the animals is as close as is to be found in comparative studies of any other structure common to all of the individuals in this group. While it is admitted that a certain amount of variation is to be found in the nuclei in the various forms of animal life, and even on the two sides of the same animal, these variations, in normal tissue, were found to be confined within fairly narrow limits.

With these facts in view, therefore, we would expect to find, and do find, the inferior olive presenting one definite and constant type both in its gross and minute structure, while the superior olive presents quite another type. While they may be said to possess some general features in common, just as is true of any two nuclear masses, they nevertheless differ so widely in form, degree of complexity, folding, size, relations, cell and fiber-content, that they can be classed only as separate and distinct entities. In comparing the two in the different species an interesting feature

develops. The simplest type of the superior olive was found in man, the most complex in the cat and rabbit. In the inferior olive these conditions are reversed, the most complex nucleus being found in man, the simpler types in the lower animals.

The pontine olive in most animals consists of two portions; a smaller, medially placed, bar-shaped mass, and a larger, laterally placed, S-shaped portion. The surfaces of these cell collections, seen *en masse*, are only slightly irregular, lacking entirely the crinkled, tortuous outline which is so marked in the larger, bulbar olive. The nucleus is entirely surrounded by a very rich network of fine nerve fibers, which is in close relationship with the transverse fibers of the corpus trapezoidum, but there is no demonstrable intranuclear commissure between the olives of the two sides, such as is found so well developed in the medulla—the interolivary decussation. There seem to be no cross fibers aside from those to be identified with the corpus trapezoidum. The individual sections of both the medial and lateral portions of the superior olive are much broader and more robust than is the shell of the inferior nucleus, and show a much more densely packed mass of cells.

Kölliker, in his embryology, gives the most complete and accurate description of the superior olive extant. He states:

“One can get a better understanding of the relations of this olive from the lower mammalia, where this organ is better developed than in man. . . . Whether this olive appears simpler or more complex, it always possesses essentially the same structure in man and the lower animals, as Golgi's slides of young animals show.”

With both of these statements my own findings are in entire accord, though Kölliker gives no results other than those obtained from a study of individual sections, and hence leaves much to be desired as to the gross morphology of this important nucleus.

A detailed study of the models of the upper olive in the three subjects chosen as the basis for this paper shows the general similarity of outline, as stated above; that of the cat being the most complex, the human the simplest, and the dog constituting an intermediate. The nucleus of the adult cat presents a medially situated, bar-shaped mass which is separate and distinct from a laterally placed S-shaped mass. The latter is considerably less extensive vertically than is the former, being overlapped from above by the outward-leaning medial mass. Both portions of the nucleus are smooth in outline, rather comparable to the accessory mass of the inferior olive, and not at all resembling the wavy, crumpled outline of the major nucleus.

The medial mass in individual sections begins about 16 sections farther caudalward than does the lateral mass. It is rather club-shaped, curving somewhat like an italic letter *f*, its extremity pointing ventro-laterally and dorso-medially, the dorsally directed pole being slightly broader than the other. The highest level reached by this medial portion is 38 sections farther cerebralward than the highest point of the lateral mass. The medial bar trends gradually lateralward, farther from the median raphé, and becomes thicker and more robust as we ascend the stem. The model of this medial portion is a long, band-like column occupying about the lower half of the pons. It is somewhat irregular, presenting a wavy outline from above downward, with a decided lateral inclination, so marked above

that it completely overhangs the medial portion of the lateral mass. Figure 7, plate 2, shows the relative positions of the nuclear complex in transverse section, and figures 2 and 3, plate 1, show the model of the entire nucleus, the two portions having been pinned together in the piling, in order that their proper relations might be retained. The model of the medial mass shows an antero-posterior concavity laterally directed, which corresponds to the rounded medial portion of the lateral mass, the two being separated by a narrow interval occupied by the fiber bundles, all of which appear to run parallel to the long diameter of the nuclear masses that bound them in transsection.

The laterally placed mass is more complex and of considerably greater bulk than is the medial portion. Beginning caudally as an irregular cell mass, this lateral portion soon assumes the typical S-shaped double curve, the medial bar of the S pointing ventralward, the lateral bar dorsalward. Thus there are two hila, a medial one opening ventrally, a lateral one opening dorsally, the lateral limb of the S and its ventral coil being shorter and more robust than the medial one, the bar of which is slender and elongated. Its ventral tips in successive sections constitute the most irregular portion of the entire nuclear mass. At certain levels, corresponding to the more atypical portions to be found at the caudal pole of the mass, there are numerous small, irregularly shaped cell-masses, situated in the area between the ventral extremities of the medial bar and the medial limb of the lateral mass, giving the impression that there had at one time been a continuity of structure which had been severed by the fiber-bundles so numerous in this area. The surface of the lateral mass is smooth and is more regular in outline than that of the medial bar. The thick lateral coil, which is ventrally directed, ends abruptly as a wide cell-mass at a point somewhat cephalad to the mid-point of the general nuclear mass; whereas the less robust medial coil, which is dorsally directed, extends farther cerebralward, becoming progressively smaller and ending in a blunt, rounded point almost in contact with the shelving lateral surface of the medial bar at the junction of its caudal three-fourths and cerebral one-fourth. This medial portion of the lateral mass is completely roofed in by the broad upper portion of the medial mass.

The superior olivary nucleus in the dog presents the same general features as those found in the cat, though lacking in some degree the symmetrical outline which is so marked in the feline nucleus. The canine superior olive also consists of two portions, a medial and a lateral portion, which are continuous for a short distance toward the caudal pole of the nucleus, where there is less regularity than is noted elsewhere in the nuclear mass. The medial mass begins caudally at a somewhat lower level than the lateral, usually one or two sections. Comma-shaped at first, it speedily becomes racquet-shaped, the handle of the racquet pointing lateralward (fig. 8, plate 2), and the larger portion thinning out in the center until it consists of a central space surrounded by a narrow coil of cells. The lateral portion of the coil in turn thins out and disappears as the summit of the mass is approached; thus the coil becomes U-shaped in its cerebral one-fifth, the hilum of the U looking lateralward. The central cavity extends fully half-way down the cell-mass. The medial mass is perfectly smooth and regular in its upper one-fourth;

below this it presents the irregular, wavy outline noticed in the other nuclei. In its lower fourth it is markedly irregular, and above the caudal pole its lateral extremity is continuous for a short distance with the ventral extremity of the medial limb of the S-shaped lateral mass, as shown in figure 2. The outline of the medial mass is distinctly rounded except laterally, where it presents in its lower three-fifths a decided antero-posterior concavity to conform to the rounded medial portion of the lateral mass, as was noted in the cat. While the upper portion of this medial mass extends farther lateralward, there is no tendency to overhang the lateral mass, from which it is separated by a narrow, fiber-filled interspace, except at the point where the two masses are continuous, as described above.

The lateral mass is bulky and S-shaped, and, while lacking the beautiful regularity of the cat specimen, presents the ventrally directed medial limb and the dorsally directed lateral limb of the S, and two hila—a medial one, ventrally directed, and a lateral one dorsally directed. The two portions of this lateral mass are coextensive in vertical measurement, the whole coming rather short of the cerebral fourth of the medial mass.

In the adult dog, owing to the peculiar histologic structure of the superior olive, the relatively small number of cells, and the rich fibrillar network, the outlines of the nuclear mass are much less distinct, especially within the nucleus where the hilum is but faintly defined, making it difficult to distinguish the exact line of demarcation. The peripheral limits are brought out more clearly by the encircling coils of fibers, which everywhere separate the nucleus from the surrounding structures. The hila are very narrow, and the fibers within them are very delicate and not so numerous as in cat and human tissues, nor do they take the stain as kindly. The nuclear fold is coarser and more loosely woven, being much wider from hilum to periphery, and the loose meshwork contains a great deal of granular material.

Compared with the nucleus in the dog fetus, we find the general outlines to be nearly identical. In the adult, however, with its greater area in cross-section with relatively little increase in the cell-content, there is a disproportionately greater increase in the richness of the fiber-network. This would lead to the assumption that the fiber element proper to a given nucleus is a large, if not the most important factor, in determining the form of the nuclear mass, which is somewhat more complex in the fully developed adult animal. The only modifying element apparent is the great number of relatively large sized blood-vessels which channel the nucleus in every direction.

The detailed study of the superior olive in man showed a much simpler picture than was found in either the cat or dog, as will be seen in figures 2 and 3, plate 1. The nuclear mass as a whole is relatively smaller than in either of these animals. It differs from them also in shape and in the disposition of its component cell masses, the human nucleus consisting of three separate and distinct portions instead of two. The medial portion is well developed and essentially like that in other animals. The lateral portions in the specimen figured herein consist of two cell-masses, one above the other, and separated by a small interval; the two, however, sustain the same general relations to the medial mass and to the surrounding parts



as is characteristic of the more fully developed lateral masses. There was considerable variation in the size and form of the lateral portion of this nucleus in the human material examined, though in no case was it entirely absent. Other investigators appear to have had similar experiences. Kölliker, whose report is rather confusing in some respects, since it was based entirely upon a study of individual sections rather than upon reconstructions, states that in cross sections of this nucleus one can, as a rule, distinguish three portions more or less distinctly: a larger, medial, more tape-like portion, and two ventral, lateral, more cylindrical formations; yet in man the resemblance to the folded membrane of the larger olive is entirely lacking. Weed, who includes a description of the superior olive in his study of the human brain-stem, says:

"The nucleus olivaris superior begins just caudally to the middle of the nucleus nervi facialis and, sloping dorsally and slightly laterally, terminates cephalad in the region of the sensory enlargement of the nervus trigeminus. . . . The nucleus enlarges into a triangular nuclear mass, out of which three dorso-ventral cell columns appear clearly defined. These are united at their ventral aspect and they spread out from this ventral point like spokes from a hub. . . . The mesial column arises from the ventral point of radiation in the caudal cell collection as a small continuous cell-mass. This . . . enlarges into a thin sheet of cells which run cephalad. . . . This sheet of cells lies in a general dorso-ventral plane, but its ventral border is placed more laterally from the mid-line than its dorsal margin. . . . The lateral of the two cell-columns is really double throughout the middle portion of its extent, although it arises singly from the mesial surface of the dorsal union of the three primary radiate columns. Arising from this union, the column extends as a triangular cell-column, placed dorsally and somewhat mesially to the dorsal border of the mesial column. . . . At this point (the level of the superior pole of the seventh nucleus) the cell-column bends laterally and dorsally across the superior pole of the olive and then pursues a cephalo-lateral course to fuse quickly with its second portion."

This second portion he describes as arising caudal to the superior pole of the seventh nucleus and ascending, as an elongated oval with the long axis in the dorso-ventral plane, to join the first portion as stated.

Judging from these statements one must conclude that there is considerable variation in the different specimens examined. One point, however, seems clear; *i. e.*, whatever the condition of the other nuclear parts, this medial portion is always present and well developed, and in comparison with the lateral mass presents a preponderance of the typical spindle-cells. All this tends to confirm the assumption that this portion is the more essential part of the nuclear complex, at least for the proper performance of the functions common to all the types under consideration.

The medial mass is a large, band-like column of cells which begins caudally just above the ponto-bulbar junction and extends forward to the region of the nervus trigeminus, exceeding the vertical limits of the lateral portion at both poles. It has a decidedly dorso-lateral inclination from below upward and is somewhat twisted upon itself in its long axis. Beginning below as a small oval mass, it speedily enlarges in both lateral and antero-posterior directions and, when fully developed, is semilunar in outline on cross-section, being convex medially and concave laterally, one extremity of the demilune pointing ventro-laterally, the other dorso-laterally,

the latter approaching the median line. The shifting in position of the successive sections, confirmed by careful measurements, involves changes in two directions—the rapid, dorso-lateral inclination, which *in toto* attains a considerable degree, the topmost section being at an angle of 45 degrees with the perpendicular of the most caudal section, and a twisting upon its caudal pole to such an extent that the extremities of the bar swing around a quarter of a circle, the long diameter of the individual section extending almost transversely. The entire mass forms a robust cell-column of irregular, wavy outline in a cephalo-caudal direction, more marked upon the convex ventro-mesial surface. The dorso-lateral surface is concave, conforming to the adjacent medially directed surfaces of the lateral masses and overhanging the upper lateral mass to a certain extent above.

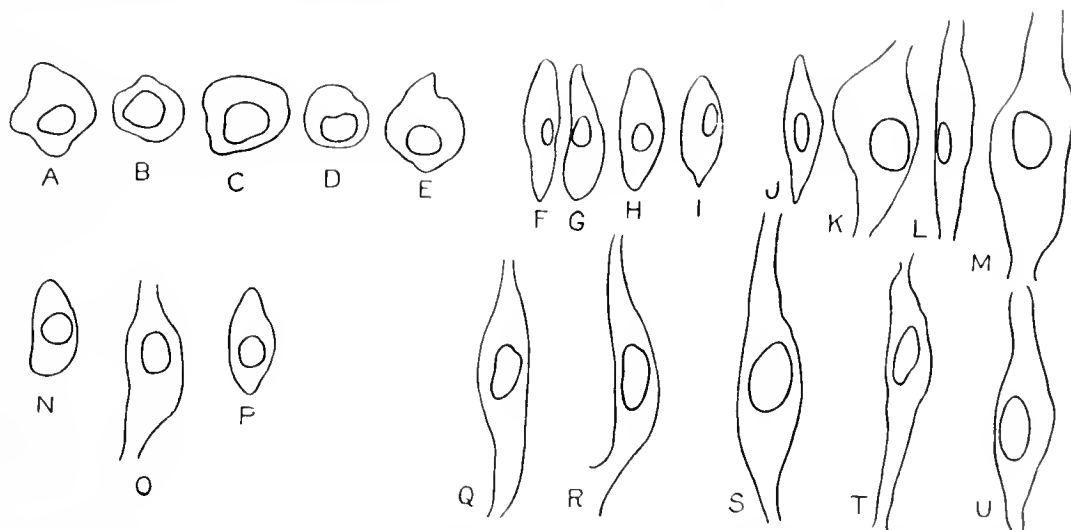
The lateral mass is much less developed in man and consists of two portions, one just above the other. The lower, slightly larger portion, begins 19 sections above the caudal limit of the medial bar and extends somewhat above the mid-point of this bar, where it ends in a pointed extremity. The second mass begins 5 sections cephalad to the former and ends in a blunt pole 21 sections below the cerebral limit of the medial bar. These lateral portions are simply irregular cell-masses presenting no hila and no definite modeling comparable to the beautiful S-shaped structure found in other animals. Both masses are channeled by blood-vessels, and are situated very close to the medial bar, almost in contact with its dorsally directed margin; whereas the point of continuity in the dog and that of closest proximity in the cat are with the ventral margins of the two portions.

#### CELL STUDY.

The superior olivary nucleus presents an enormous number of nerve-cells which have been variously described by different authors, the majority of whom, however, are perhaps agreed that the cells in the superior and inferior olives are alike. This is well demonstrated in Barker's summary, which is to the effect that Held, Kölliker, and Cajal describe their results in a general way as follows: The cell bodies in the superior olivary nucleus markedly resemble in type those found in the inferior olive and the dentate nucleus; they possess numerous, much branched dendrites which are turned toward the interior of the nucleus, the axons in the main being directed toward the periphery of the nucleus. Still others describe these cells as being spindle-shaped or club-shaped. One reason for this diversity of opinion, perhaps, is the fact that prior to complete development and differentiation the great majority of nerve cells resemble each other rather closely, since all are of the embryonic type; and in studying a given cell-mass in an embryo, even one comparatively well advanced in other respects, one is liable to be led into error as to the definite morphology of a given type of cell unless one studies the same cell area through several successive stages of development. It is even more satisfactory to study the fully differentiated adult stage as well. A much more complete picture of a given cell group can be obtained by comparative study in a number of animals.

In a comparative study of the inferior olive (unpublished) it was found that there was a striking uniformity in shape, size, and structure of the cell peculiar to

this nucleus. There is also, of course, a similarity in the gross morphological features of the nuclear masses as well, but the conformity to type of the gross outline is neither so striking nor so significant as is the likeness of the cells characteristic of this nucleus. These features were noted throughout the series of animals used in that study. The same morphologic similarity obtains in the case of the superior olivary nucleus, both for the mass of the nucleus and more particularly for its constituent cells. It will be found that when complete adult differentiation has been attained these two groups of cells—those of the inferior and those of the superior olive—will each have developed into a type of cell peculiar to and characteristic of the given collection or nuclear mass within which it is found, each type being sufficiently definite as to morphology to enable the investigator to positively determine its proper repository without difficulty, as shown in figure 1, a camera-lucida tracing of the various cells under discussion. (Compare these with the microphotographs of the different sections, figs. 4, 5, and 6, plate 1).



TEXT-FIGURE 1.—Outline tracings of the cells in the two olives; cells A-E inclusive are from the human inferior olive; cells F-I are typical cells from the superior olive of an adult dog; cells J-M are from the medial mass of the superior olive of an adult cat; cells N-P are from the lateral mass of the same; cells Q-U are typical cells from the superior olive of a human fetus. The outlines were made with camera lucida, A-P being made with a 4 mm. objective and No. X ocular, Q-U being made with a 1/12 objective and No. X ocular.

The cell type which is characteristic of the superior olive is more or less definitely spindle-shaped, a great number being true spindles with a narrow, long-drawn-out cell body, the nucleus being compressed laterally (ovoid) to conform to the shape of the cell body, and the processes coming off from the two extremities of the spindle. Another type of cell, especially abundant in the olive of the adult cat, presents a large cell body with a large vesicular nucleus, the cell body tapering sharply at its two poles. Unless studied carefully through a series of sections, these cells may be thought to be club-shaped. Indeed, Kölliker classes them as such. A careful serial study, however, shows them to present two sharp poles with a rather fat intervening body, a modified spindle. A third type of cell found in but not limited to the superior olivary nucleus in all animals is a small, rounded,

granular cell, scattered irregularly through the nuclear area and the surrounding parts. The spindle-cells are closely packed, side by side (like small fish in a tin), their extremities pointing toward the periphery of the nuclear fold, the long axis of the cell being placed at right angles to the long axis of the nuclear coil. This condition is especially apparent and regular in the medial mass of the nucleus, as this portion corresponds more nearly to a straight line than does the S-shaped lateral mass, though in the latter the spindles extend crosswise of the fold, thus conforming to type.

Some of the cells present irregularities in outline, though all are sufficiently close to type to justify one in classifying them as belonging to the same general cell family which is characteristic of this nuclear mass. The different animals exhibit some minor variations. In the adult dog, for example, in proportion to the fiber network, the cells are less numerous than in other animals, and thus the difference between the spindles and the tapering oval cells is more clearly defined. The spindles are quite deeply stained, while the tapering, oval cells are very pale and rather indefinite. In the cat the staining reactions are similar, though the ovoid cells are much more in evidence and take the stain better, and the total cell-content is vastly greater than in the dog.

In human tissue, aside from a few granular cells, only true spindle-cells are found. These present the characteristic shape, position, and relations as those described for other animals. The cells are deeply staining, very numerous, and closely packed together. The medial bar, which is the more constant and essential portion of the nuclear complex, presents these cell conditions to the best advantage. These facts, coupled with the finding of great numbers of true spindles to the practical exclusion of other cell types, strengthen the assumption that the spindle-cell is the one peculiar to and characteristic of this nucleus. The lateral masses show the same general cell characteristics, though they are not as regular in the medial mass. While the nucleus is very richly supplied with blood-vessels in all the animal forms, the human nucleus is especially vascular.

A careful study was made of the cells in the areas adjacent to the superior olive in order to definitely delimit the nuclear mass under consideration. There are several groups of these cells to be considered, and for purposes of description these may be classed as follows: (1) The cells of the nucleus facialis, which, though situated in the *formatio reticularis*, for obvious reasons require specific consideration; (2) the cells in the *formatio reticularis*; (3) the cells in the *corpus trapezoideum*.

(1) The nucleus facialis is a large nuclear mass located in close proximity to the dorso-lateral surface of the superior olive, but the cells making up this nucleus are as characteristic of their kind as are those of the superior olive. These two types of cells differ so widely that confusion is hardly probable, although Weed found it difficult to differentiate the lower pole of the olive from the facial nucleus in the tissue which he studied. A study of the fiber relations of these cells is also an aid in distinguishing them.

(2) The *formatio reticularis* presents a great number of cells which are, for the most part, scattered irregularly throughout the brain-stem. At times a few

of these cells will be found grouped together, though at no place constituting definite nuclear masses save such as have long since been identified and named. While in every section examined some of these scattered cells were found in close relation to the superior olive, in no case is it especially difficult to exclude them from the make-up of the nucleus proper, since morphologically they differ widely from those proper to the olive.

(3) There are a great number of cells in the area of transverse fibers ventral to the superior olive. The difference in the disposition of the cells comprising the nucleus pontis found in the lower animals as compared with man, makes their study rather more difficult, and it becomes quite a problem (in the human as well as in the animal forms) to separate the scattered cells into two classes, one to be designated as the nucleus pontis and a second as constituting certain smaller nuclei which have been described as being closely associated with the superior olive. A number of these nuclear masses, such as the nucleus preolivaris, the nucleus semilunaris, and the nucleus corpus trapezoideum, will be discussed in a subsequent paper. In this connection we find, in the lower animals especially, that the great majority of these cells are grouped within a rather limited area adjacent to the ventral surface of the superior olive, although evidently not forming a part of this nuclear complex, since these cells have, for the most part, rather large, irregularly rounded, darkly staining cell-bodies with deeply staining nuclei. In this they differ widely from the typical olivary cell.

There is a quite noticeable cell group to be found at some levels, in cat tissue especially, immediately ventro-mesial to the medial mass of the olive; and in one area, extending over several sections, there are numerous irregular clumps of these cells between the ventrally directed medial limb of the S and the ventral pole of the medial mass, surrounded by streams of fibers. Thus, in a sense, they seem to piece out a connection between the two portions of the major nuclear complex. These groups, and others less numerous, found along the ventral surface of the olive, may be genetically related to the olivary cells, though there is but the faintest morphological resemblance. There are also many fine collaterals passing between the trapezoidal fibers and the olivary cells proper, which stream around and among these cells and possibly establish relations with them.

Since, as has been noted above, the medial nuclear mass is well represented in every animal and, conforming more closely to type, is the more constant portion of the nucleus; and as the spindle cell has been seen to be the type of cell characteristic of the superior olive, we find, as would be expected, additional support for the idea of the conformity of structure to function in the fact that the best-defined and the greatest number of spindles are found in the medial nuclear mass, which may therefore be assumed to be the most essential part of this nuclear complex.

#### THE RELATED FIBER ELEMENT.

There are great numbers of nerve cells in the superior olive. Associated with these is a correspondingly rich network of both afferent and efferent nerve fibers. These, as Cajal observed, are so numerous and so exceedingly fine and the network

so intricate as to render their study a very difficult task. Much work has been done in an effort to determine the fiber relations of this portion of the brain-stem, and certain facts have been fairly definitely established; among others, the manner in which the fiber element of the superior olive is related to the corpus trapezoideum, the lemniscus lateralis, and the tractus olivo-nucleus abducentis, and through these indirectly to the various parts with which these tracts are connected. While it is impossible at this time to give a complete study of these fiber elements and their relations to other parts of the central nervous system, a few of the more pertinent facts may be recorded.

The fibers that are distinguishable in relation to the superior olive may be roughly grouped into three classes: (1) Those that can be traced from the olive to the nucleus of the abducent nerve, the olivary peduncle (shown in figs. 7 and 10, plate 2). These fibers are quite fine and are grouped in small bundles which can be traced vertically from the dorsal surfaces of both medial and lateral masses of the superior olive, from a point near its middle portion to the nucleus nervi abducentis. The bundles run parallel to the emergent portion of the nervus facialis. The olivary peduncle forms a connecting link between the auditory pathway and the nerve supply of the ocular muscles. Santee believes that a part of the fibers from the olivary peduncle go to the nucleus abducentis, and part go by way of the medial longitudinal bundle to the trochlear and oculo-motor nuclei, thus correlating all the nerve nuclei supplying the ocular group of muscles with the auditory pathway. This may serve to explain some of the results of Ferrier's experiments upon monkeys. This observer claims that the animals will, when anesthetized, turn the eye in the direction from which a sound is perceived, a similar movement being observed when the cortical center for hearing is stimulated. This action will necessarily call into play the lateral rectus muscle through stimulation of the nervus abducentis.

(2) This group would include all those fibers which extend between the ventrally situated corpus trapezoideum and the ventral surfaces of both parts of the nuclear mass of the superior olive. These vary in length; some can be traced for only a short distance, others extend well out toward the periphery of the section. All are better developed in adult tissue. Some of the fibers are terminal, but the major part is undoubtedly collateral. In the trapezium they can be seen to branch, T-shaped, one collateral passing dorsalward toward the olive. The olive rests in a bay in the dorsal surface of the corpus trapezoideum, the fibers of which curve around ventral to the nucleus. The strands of trapezoidal fibers are observed to occupy a much greater antero-posterior extent in the interval between the olives of the two sides (fig. 5), but curve sharply forward at the medial margin of the nucleus to decussate in the median line with those from the opposite side, none of them apparently entering the nuclear mass. Indeed, one commonly finds the collaterals, not the terminal fibers, entering the nucleus, though the fibers of these strands appear to give off no collaterals in this region. Kölliker claimed to have found, in a study of frontal sections, fibers coming from the direction of the median raphe to end about the cells in the dorsal part of the olive. Barker suggests that

the olive also receives fibers from the *formatio reticularis*. It is entirely probable that both collaterals and terminals pass in both directions between the superior olive, a way-station in a specific tract, and that the cells in the *formatio reticularis* constitute a part of the general association system.

(3) This group includes the fine fibers surrounding the periphery of the nucleus in well-defined bundles and filling the interval between the medial and lateral masses, and also the hila of the lateral mass. Most of these fibers are probably axons of the cells making up this nucleus. While fibers are seen to turn into the substance of the cell-mass at all parts and levels, no bundles can be observed to cut into and through the substance of the nuclear fold, which is so noticeable in the fiber arrangement of the inferior olive; though in the cat the medial mass appears at some points to have been separated from the lateral mass ventrally, and a still greater tendency to group into bundles has been noted in the fibers of human tissue.

Cajal describes the axons arising from the cells in the superior olive as passing in three different directions: (1) The majority of them, after giving off collaterals in the nucleus itself, pass to the dorsal surface of the nucleus, where either by bending or bifurcating they turn to run vertically in a longitudinal bundle continuous with the lateral lemniscus of the same side. (2) A certain number of the axons, much curved inside of the nucleus, leave the latter at its lateral border to enter the trapezoid body, where they can be followed nearly as far as the ventral cochlear nucleus. (Held describes these as actually terminating inside of this nucleus.) (3) Other axons, arising from the cells of the superior olive, pass out at the medial side of the nucleus, entering the plexus of the preolivary nucleus to mingle there with the trapezoidal fibers. Held adds to these the group of axons which make up the olivary peduncle described above.

It is to be noted that the number of these fibers is greatly augmented in the upper part of the nucleus. In the cat specimen, especially, great, dense whorls of fibers are seen around and above the superior pole of the olive, where they form the beginning of the lemniscus lateralis. In none of the tissues studied could any connection be found between the cell-mass of the superior olive and the nucleus of the lateral lemniscus, though such a condition is claimed by some investigators. Bruce asserts (p. 48) that these two nuclei are continuous, and Cajal states that the lower nucleus of the lateral lemniscus is anatomically continuous with the superior olive. Nevertheless, it is to be sharply separated from the latter, for its constituent cells are very different in shape and the axons are entirely different in distribution.

The cell and fiber complex in the adult dog presents some features which are altogether different from those observed in the other forms studied, there being a disproportionately greater amount of fibrillar element as compared with the other animals, the fine intranuclear fibrillar network making up the bulk of the nuclear mass, the cells being widely scattered and enmeshed in the plexus. The extra-nuclear fibers surrounding the nuclear mass are less abundant than in the cat. The individual section of the superior olive of the dog is much coarser, presenting a greater transverse measurement and a less sharply defined hilum. The adult cat is the best subject for fiber study. Quite bulky strands of fine fibers can be seen

flowing around the periphery of the nucleus and in the interval between the medial and lateral masses, filling in the hila and forming dense whorls around the superior pole, making altogether a much richer network than is found in any other form.

In man there is a well-developed fiber complex, both intranuclear and extra-nuclear; the latter, in particular, shows a tendency to group into bundles. These can be seen at places cutting the substance of the nuclear mass in the same manner, though in much less degree, than is commonly found in the inferior olive.

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#### DESCRIPTION OF PLATES.

##### PLATE 1.

- FIG. 2.—Photograph of the ventral aspect of three models of the superior olive: A, adult cat; B, dog fetus; C, human fetus. A and B are oriented to conform to their natural position in the stem. C has been placed to show the relations of its different parts to each other; the picture accordingly shows the ventro-lateral view of the vertically placed nucleus, whereas in its proper position it should lean sharply to the right with some twisting upon its long axis. In A and B the S-shaped lateral mass can be clearly seen, separate from the medial bar in A (cat nucleus) but continuous with it in the lower part for a short distance ventrally in B.
- FIG. 3.—Dorsal view of the same models. In A the interval between the two masses is well shown. In B the marked irregularity of the lateral bar of the S and the absence of continuity of the two masses are noted. In C the three component portions are clearly shown, though the interval between the medial and lateral masses is slight.
- FIG. 4.—High power microphotograph of the superior olive in an adult cat, showing the characteristic spindle-cells. All of the sections in figures 4-6 were cut 20 microns and are too thick to give sharp pictures.
- FIG. 5.—Spindle-cell in the adult dog.
- FIG. 6.—High-power photograph of the human superior olive in embryo No. 28, in which the closely packed arrangement and large number of spindle-cells are evident. In all of these the same character of cell (the typical spindle) will be found.

##### PLATE 2.

- FIG. 7.—Lower power microphotograph of a section of the pons of an adult cat. Here the superior olive can be seen to consist of a bar-shaped medial portion and an S-shaped lateral portion, together forming a compact mass lying on the dorsal surface of the trapezoid body between the emergent fibers of the abducent and facial nerves.
- FIG. 8.—Microphotograph of the superior olivary region in a 115 mm. dog fetus. The olive consists of a racquet-shaped medial portion and an S-shaped lateral portion. The cluster of large cells dorsal to the latter is the nucleus of origin of the facial nerve.
- FIG. 9.—Microphotograph of a Yamagiwa preparation of the brain-stem of an adult dog, showing the C-shaped medial portion of the superior olive with the emergent fibers of the abducent nerve passing through it. The lateral portion is O-shaped.
- FIG. 10.—Photograph of a Pal-Weigert preparation in the region of the superior olive in a new-born infant, showing the fiber elements. To the right of the section is the thick strand of fibers of the facial nerve, and just medial to this is its nucleus of origin with fibers streaming upward toward the genu. Between the facial and abducent nerves is the superior olive, lying medial and ventral to the nucleus of the facial. The fine fibers streaming dorsally from it constitute the olivary peduncle.





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A

B

C

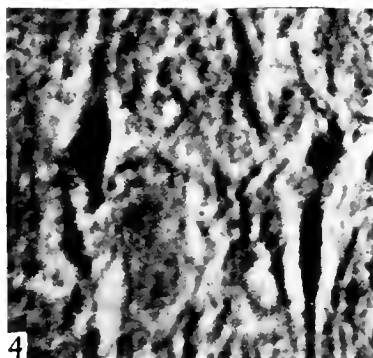


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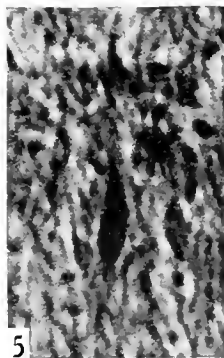
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B

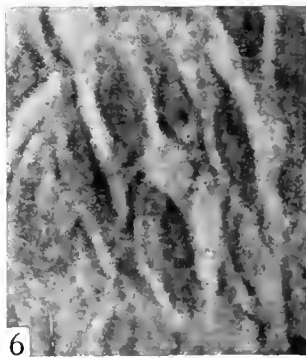
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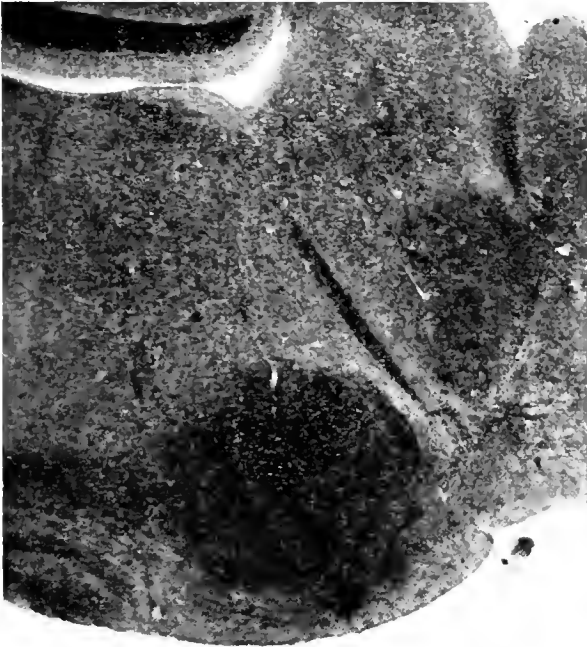


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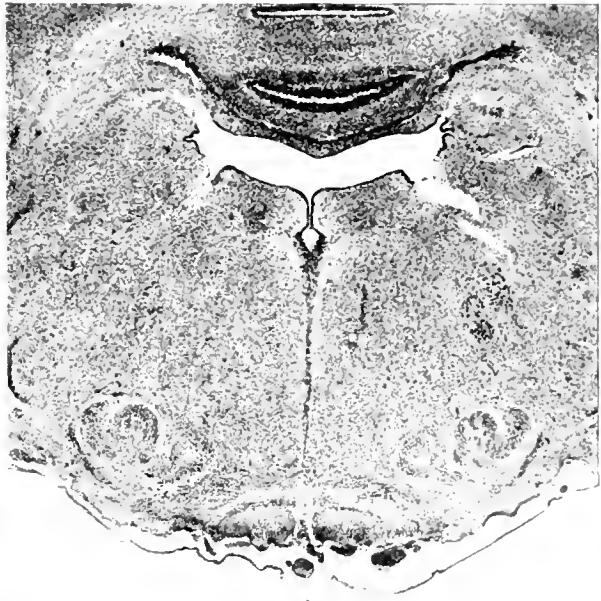


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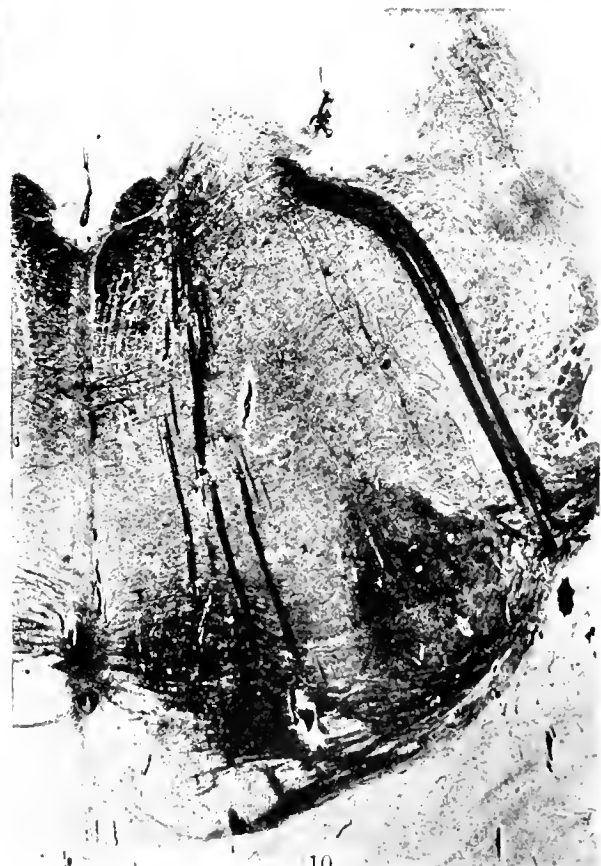
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CONTRIBUTIONS TO EMBRYOLOGY, No. 34.

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THE DEVELOPMENT OF THE EXTERNAL NOSE IN WHITES  
AND NEGROES.

BY ADOLPH H. SCHULTZ,

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With seven figures and one plate.

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# THE DEVELOPMENT OF THE EXTERNAL NOSE IN WHITES AND NEGROES.

BY ADOLPH H. SCHULTZ.

The human nose, in the adult stage, furnishes one of the most important differential characteristics of the various races. The question as to how early in its development this racial difference becomes apparent is treated herein, although the main purpose of the study has been to add something to our knowledge of conditions of growth of the external nose.

## MATERIAL.

The material used as the basis of the study consisted of 320 fetuses of the Carnegie Embryological Collection, all of which were normal specimens in good condition. The great majority of them had been preserved in 10 per cent formalin, the remainder in 80 per cent alcohol or 3 per cent carbolie acid. The fetuses range in age from the tenth week of pregnancy to full-term. The former age was chosen as the minimum because younger fetuses could not be measured with the same instruments used for those of more advanced development, nor could the same technique be applied. Of the specimens 254 were whites, 58 American negroes, 4 Filipinos, 3 Indians, and 1 Japanese. In a very few cases no data concerning the race could be obtained; these specimens, however, were recognized to be white on the basis of a careful metric comparison with material of known origin, and in every instance the evidence was perfectly conclusive. The determination of the age was based on the sitting height, according to Keibel and Mall (1910). For the purpose of comparison measurements analogous to those taken on fetuses were made on the bodies of 8 children and 35 adults.

Table 1 shows the numerical distribution of the material in reference to age. Fetal material of sufficient quantity for a classification according to weeks was available only up to the end of the fifth month. Specimens from the sixth month were classified in two groups, while those from the later months of intrauterine life constituted such a small minority that they were considered without regard to weekly divisions. The average sitting heights of whites and negroes of corresponding age are frequently somewhat at variance. Relatively speaking, however, these differences are negligible.

TABLE 1.—Numbers of specimens according to race and age.

Age.	Number of individuals.		Average sitting height (mm.)	
	Whites.	Negroes.	Whites.	Negroes.
10th week.....	8	.....	39	.....
11th week.....	15	4	48	48
12th week.....	25	2	62	58
13th week.....	26	3	76	76
14th week.....	43	4	91	93
15th week.....	21	4	106	104
16th week.....	18	2	116	115
17th week.....	25	9	128	128
18th week.....	11	2	142	138
19th week.....	13	2	153	151
20th week.....	10	2	161	164
21st and 22d week	14	4	177	181
23d and 24th week	6	3	203	202
7th month.....	6	3	224	228
8th month.....	4	5	259	262
9th month.....	2	4	292	300
10th month.....	7	5	343	341
Children.....	.....	8	.....	.....
Adults.....	10	25	.....	.....

## TECHNIQUE.

The following measurements (in millimeters) were taken on each specimen (see fig. 1). The sliding compass of Martin was used for measuring or (in the case of small specimens) a fine pair of dividers and a metal ruler.

**Sitting height:** The fetus is placed on its back upon a horizontal surface, with the thighs and the ear-eye horizon at right angles. The distance between the vertex of the head and the most caudal point of the gluteal region, parallel to the median-sagittal plane and to the horizontal surface, constitutes the sitting height.

**Upper-face height:** Distance between nasion and stomion. The nasion on the face is situated horizontally in front of the median point of the naso-frontal suture. The exact determination of this point is rather difficult on fetuses. It is always situated above the level of the eye-clefts, usually on a line uniting the highest points of the folds which limit the upper eyelids, or in rare instances even slightly above. This was determined by dissections and examinations of numerous median sagittal sections of fetal heads. With but few exceptions the nasion was found to be above the deepest depression of the nasal bridge, a relation which holds good also for adults in the majority of cases (Schultz, 1918). The stomion is the median point of the oral cleft. In the not infrequent cases where a tuberculum labii superioris projected downward the stomion was placed at the base of this—i. e., the tuberculum was not included in the upper-face height.

**Nasal height:** Distance between nasion and subnasale point. The subnasale is situated in the median sagittal plane, where the nasal septum meets the upper lip. In very young fetuses the latter two have almost the same direction and the determination of this point of measurement is therefore in that stage only approximate.<sup>1</sup>

**Nasal breadth (lower nasal breadth):** Greatest width between the alæ nasi.

**Interocular breadth (upper nasal breadth):** Width between the inner canthi.

**Interzygomatic breadth:** Greatest width between the zygomatic arches.

**Breadth of the nasal septum:** Smallest distance between the nostrils.

Two angles of the nose were measured with the aid of a three-legged divider, which proved to be a very useful instrument.<sup>2</sup>

**Vertical nasal angle:** The three points of the dividers are placed on the nasion, the apex of the nose and on the subnasal point; the divider is then carefully set on a piece of paper and slightly pressed down. The three points of impression thus obtained are united by straight pencil lines (nasion to apex of nose, and from the latter to subnasale) when the angle between these lines is measured.

**Horizontal nasal angle:** The three points of the dividers touch the most posterior point of the ala nasi of each side and the apex of the nose, after which the divider is again transferred to paper and so on.

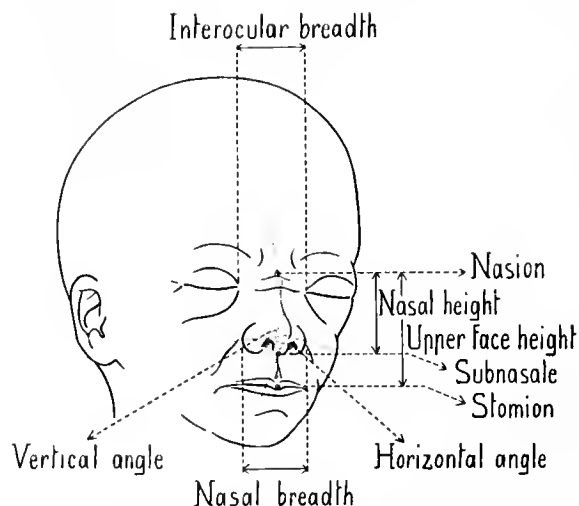


FIGURE 1.—Schematic representation of a fetal head, with measurements and points of measurement.

<sup>1</sup>The writer would suggest that those interested in studies of this question begin on older fetuses in order to secure the degree of experience necessary for the purpose of studying younger specimens.

<sup>2</sup>A three-legged divider of different construction was used by Mérejkowsky ('82) for measuring the elevation of the nasal bones.



From the absolute measurements a number of relative ones, or indices, have been obtained according to the following formulæ:

$$\text{Relative nasal height: } \frac{\text{nasal height}}{\text{upper face height}} \times 100.$$

$$\text{Relative nasal breadth: } \frac{\text{nasal breadth}}{\text{interzygomatic breadth}} \times 100.$$

$$\text{Nasal index: } \frac{\text{nasal breadth}}{\text{nasal height}} \times 100.$$

$$\text{Upper facial index: } \frac{\text{upper face height}}{\text{interzygomatic breadth}} \times 100.$$

$$\text{Relative interocular breadth: } \frac{\text{interocular breadth}}{\text{interzygomatic breadth}} \times 100.$$

$$\text{Lower to upper nasal breadth: } \frac{\text{nasal breadth}}{\text{interocular breadth}} \times 100.$$

### THE HEIGHT OF THE NOSE.

Table 2 is a compilation of the averages and ranges of variation of the nasal height, upper-face height, and relative nasal height in the different age groups. To calculate standard deviations and variation coefficients for measurements on fetuses can serve no purpose, in as much as every age group represents a certain portion of intrauterine growth during which the size of the specimens increases.

TABLE 2.

Age.	Upper face height.						Nasal height.						Relative nasal height.					
	Whites.			Negroes.			Whites.			Negroes.			Whites.			Negroes.		
	Min.	Av.	Max.	Min.	Av.	Max.	Min.	Av.	Max.	Min.	Av.	Max.	Min.	Av.	Max.	Min.	Av.	Max.
10th week.....	3.9	4.15	4.8	.....	.....	.....	2.3	2.84	3.0	.....	.....	.....	59.0	68.5	75.0	.....	.....	.....
11th week.....	4.0	4.81	5.5	4.0	4.98	5.9	2.1	3.35	4.0	3.1	3.40	3.7	52.5	69.4	76.0	62.7	69.0	77.5
12th week.....	5.0	6.22	7.0	6.0	6.15	6.3	3.0	4.36	5.2	4.0	4.35	4.7	57.1	70.3	80.0	66.7	70.6	74.6
13th week.....	6.0	7.64	9.0	6.8	7.83	9.0	4.5	5.22	6.0	4.3	4.90	5.4	57.8	68.9	83.3	60.0	62.7	64.9
14th week.....	8.0	9.39	12.0	9.0	9.75	11.0	5.0	6.27	8.0	5.1	6.07	7.0	50.0	67.1	77.8	50.7	62.0	70.0
15th week.....	10.0	10.80	12.0	10.0	11.00	12.0	6.5	7.21	8.2	6.0	7.25	8.0	59.1	66.8	80.0	60.0	65.7	72.7
16th week.....	10.0	12.0	14.0	12.0	12.50	13.0	7.0	8.07	9.2	8.0	8.0	8.0	61.5	67.2	72.7	61.5	64.1	66.7
17th week.....	11.0	13.50	16.0	13.0	14.40	16.0	7.0	8.83	10.0	8.0	9.16	10.0	50.0	65.6	73.8	56.3	63.6	71.4
18th week.....	14.0	15.27	17.0	15.0	16.0	17.0	9.5	10.73	12.0	11.0	11.0	11.0	66.7	70.2	75.0	64.7	69.0	73.3
19th week.....	15.0	16.54	18.0	15.5	16.55	17.6	10.0	11.30	13.0	11.0	11.25	11.5	61.1	68.5	73.3	65.3	68.1	71.0
20th week.....	15.0	16.80	18.0	17.0	18.0	19.0	10.0	11.39	12.0	11.0	11.50	12.0	61.1	67.9	75.0	63.2	63.9	64.7
21st and 22d weeks.....	16.0	18.57	20.0	19.0	19.75	21.0	11.0	12.72	14.0	12.0	13.25	14.0	63.2	68.5	73.7	63.2	67.0	70.0
23d and 24th weeks.....	19.0	21.0	23.0	21.0	21.0	21.0	13.0	13.83	15.0	13.0	13.30	14.0	61.9	66.0	68.4	61.9	63.5	66.7
7th month.....	20.0	22.30	26.0	24.0	24.30	25.0	13.5	14.75	17.0	13.0	15.0	16.0	58.7	66.3	70.0	52.0	61.8	66.7
8th month.....	22.0	26.75	31.0	22.0	26.8	29.0	15.0	17.0	20.0	15.0	15.80	17.0	58.6	63.8	68.2	53.6	59.4	68.2
9th month.....	26.0	27.00	28.0	27.0	28.25	30.0	18.0	18.0	18.0	15.0	17.25	19.0	64.3	66.7	69.2	53.6	61.1	66.7
10th month.....	29.0	31.0	35.0	27.0	29.80	32.0	18.0	19.64	22.0	17.0	18.44	21.2	57.1	63.5	70.7	58.1	61.9	66.2
Children.....	.....	.....	.....	31.0	45.0	57.0	.....	.....	.....	20.0	28.4	36.0	.....	.....	.....	54.0	63.1	71.4
Adults.....	68.0	79.6	86.0	66.0	80.4	91.0	50.0	58.2	64.0	46.0	54.9	60.0	65.5	73.2	79.2	62.7	68.4	74.0

The deviations and coefficients would, therefore, become larger than in a group of fetuses of exactly the same age.<sup>1</sup> The range of variation of the nasal height and of the upper-face height is very considerable in whites as well as in negroes. In some

<sup>1</sup>The greatest number of specimens of the same size was found in six whites of 65 mm. sitting height; in these the nasal height varied from 4.1 to 5.0 mm.

groups of negroes the variability is apparently small, but this finds its explanation in the limited number of specimens composing these groups. For the whites of the 12th to the 17th week (all large groups) the difference in the extreme variants has been expressed in percentage of their arithmetic mean, and, in this way, a relative range of variation was obtained. Table 3 shows these relative ranges of variation for the nasal height and upper-face height. On an average these are about the same for both measurements, but may vary to a great extent in different weeks. As causes for this high variability, which will also be noted later on for other measurements, it may be mentioned, first, that the individual error in measuring increases relatively with the decrease of the size of the measurement. Inasmuch as these measurements consist chiefly of only a few millimeters, their variability is due in part to individual error. A further technical source of mistake lies in the difficulty of exact determination of the nasion. Another artificial factor which may play a rôle is the influence of the preservative. Formalin may alter different measurements on the fetal body in varying degrees. The author's investigations on this subject have not yet been concluded, but judging from previous experience it would seem rather improbable that this takes a noteworthy part in the variations of the nose. Undoubtedly the most important and influential cause lies in the natural variability of the fetal organism, which is manifest even in small fetuses and can readily be detected in preserved as well as fresh specimens.

TABLE 3.—*Relative ranges of variation.*

Week.	Nasal height.	Upper-face height.
12th....	54	33
13th....	29	40
14th....	46	40
15th....	23	18
16th....	27	33
17th....	35	37

Table 2 shows a steady increase in the average nasal height with advancing development. The relative increase, however, is rather irregular. This leads to the interesting question as to what extent the growth of the entire body influences the size of its individual parts. For the purpose of answering this question on the basis of the nasal height, the difference in the averages of two successive age groups was expressed in percentage of the smaller average—*i. e.*, that of the younger group. The figure thus obtained shows the relative increase in size during a certain period. Table 4 gives these relative increases in size of the nasal height and of the sitting height of white fetuses. Hardly any correlation exists between the relative increases in size of the two measurements. The nasal height increases relatively less during the fetal development than the sitting height; both show more intensive growth in the beginning. In the majority of the age groups the average nasal height of whites exceeds that of negroes, a relation which is opposite in the case of the upper-face height. In the latter there are only a few exceptions in which the average is greater in whites. From this we may conclude that the relation between the two measurements, *i. e.* the relative nasal height, must show a distinct racial difference, according to which the nose, as compared with the upper face, is higher in whites than in negroes. The curves in figure 2 show how this difference actually appears after the twelfth week and how constant it remains thereafter. The relative nasal height is rather variable, decreasing slightly in both races during intrauterine growth and increasing again after birth. The difference between the

relative nasal height and 100 expresses the relative height of the upper lip, the latter, as a necessary consequence of the preceding, being greater in negroes than

TABLE 4.—Relative increases in size.

Age.	Sitting height.	Nasal height.
11th week.....	23	18
12th week.....	30	30
13th week.....	22	20
14th week.....	24	20
15th week.....	17	15
16th week.....	9	12
17th week.....	10	9
18th week.....	11	21
19th week.....	7	5
20th week.....	7	1
22d week.....	8	12
24th week.....	15	9
7th month.....	10	7
8th month.....	16	15
9th month.....	13	6
10th month....	17	9

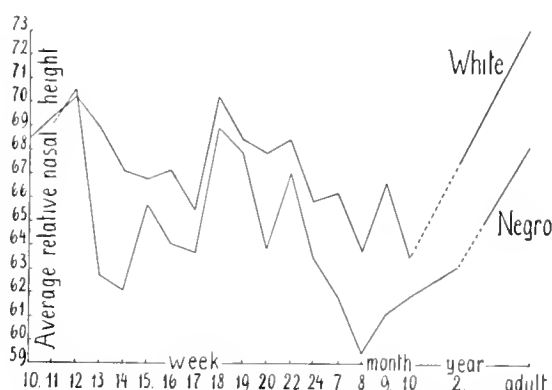


FIGURE 2.—Curves of the average relative nasal heights.

in whites. The ranges of variation of the relative nasal height in the two races overlap each other extensively; therefore this index has but little value in racial diagnosis except in extreme cases where the measurement exceeds 77.5, a number never found in negroes.

A Japanese fetus of the eleventh week had a relative nasal height of 70.5, which is about equal to the corresponding figure in whites. The Filipinos showed for this measurement the following values: eleventh week 82.5, fourteenth week 67.1, nineteenth week 63.9, and twenty-first week 57.1; these are greater in the beginning, while later they are much smaller than the corresponding figures in whites. The Indians have for this index in the sixteenth week 59.1, in the eighteenth week 76.9, and in the twentieth week 76.9, the first figure being smaller, the latter two larger than the averages of corresponding ages in whites. Unfortunately, these cases are too few to warrant any conclusions, and are merely intended to place on record the measurements obtained in these rare specimens.

Two fetuses of *Macacus cynomolgus*, sitting height 35 and 56 mm., measured by Toldt (1903), had relative nasal heights of 80.0 and 79.9 respectively, figures which are close to the upper extreme of this index in man.

#### NASAL BREADTH.

Table 5 shows the averages and ranges of variation of the absolute and relative nasal breadth. The variability of the nasal breadth is very considerable; the relative ranges of variation in whites, ascertained by the same method as was employed for the nasal height, are as shown in table 6. The average of these relative ranges of variation for the nasal breadth is 35 and for the nasal height 36. The breadth of the nose is therefore practically as variable as the height. The relative ranges of variation of both are smaller in adults than in fetuses. The 51 adult whites measured by Schwerz (1911) give a relative range of variation of 32 for the nasal height and of 30 for the nasal breadth, and the author's material of 25 adult negroes showed 26 for the nasal height and 30 for the nasal breadth. The average nasal breadth shows a steady increase with age except in the tenth month, which is

TABLE 5.

Age.	Nasal breadth.						Relative nasal breadth.					
	Whites.			Negroes.			Whites.			Negroes.		
	Min.	Av.	Max.	Min.	Av.	Max.	Min.	Av.	Max.	Min.	Av.	Max.
10th week.....	2.8	3.31	3.8	.....	.....	.....	32.0	34.5	38.9	.....	.....	.....
11th week.....	3.2	3.86	4.4	4.0	4.38	5.0	26.5	32.2	40.0	33.3	35.7	38.5
12th week.....	4.2	4.84	5.8	5.0	5.25	5.5	23.3	30.4	34.4	34.4	35.1	35.7
13th week.....	4.0	5.56	6.5	5.6	6.10	6.5	21.7	27.8	34.2	31.0	31.5	32.5
14th week.....	5.0	6.53	8.0	6.0	7.80	9.0	20.8	26.0	30.8	25.8	29.8	33.3
15th week.....	6.6	7.93	9.0	7.0	8.25	9.0	20.6	25.9	29.0	25.0	26.9	31.0
16th week.....	7.5	8.41	10.0	9.0	9.0	9.0	22.9	25.7	31.0	26.5	27.3	28.1
17th week.....	8.0	9.20	10.0	9.0	10.33	11.5	21.0	24.8	28.6	24.3	27.7	31.1
18th week.....	9.2	10.11	11.0	11.0	11.50	12.0	22.1	24.8	27.0	27.5	27.7	27.9
19th week.....	9.0	10.52	12.0	12.0	12.50	13.0	21.6	23.7	25.6	28.3	29.1	30.0
20th week.....	9.5	11.15	12.0	13.0	13.0	13.0	21.6	24.3	25.6	28.3	29.2	30.2
21st, 22d weeks.....	11.2	12.45	13.0	12.0	13.50	16.5	21.5	24.8	26.6	25.5	27.2	30.0
23d, 24th weeks.....	13.0	14.10	15.0	14.5	14.83	15.0	22.0	25.0	25.9	27.8	28.3	28.8
7th month.....	13.0	14.47	17.0	15.0	16.0	17.0	22.6	25.0	29.3	25.4	28.2	31.5
8th month.....	15.0	17.0	20.0	16.0	19.0	21.0	24.6	26.5	29.3	29.0	30.8	32.3
9th month.....	19.0	19.0	19.0	19.0	21.20	24.0	24.7	25.2	25.7	27.1	29.4	31.6
10th month.....	16.0	18.60	22.0	21.0	21.20	23.0	22.4	23.9	27.8	26.3	28.6	30.7
Children.....	.....	.....	.....	19.0	25.8	30.0	.....	.....	.....	23.7	27.2	29.6
Adults.....	31.0	35.2	40.0	37.0	42.5	50.0	23.3	25.4	28.7	27.2	31.0	35.1

unquestionably a chance occurrence and due to the small number of specimens composing this group. Using the same method employed in connection with the study of the nasal height, the relative increases in size of the nasal breadth were found to be as shown in table 7.

The relative increase in size of the nasal breadth seems to be but little correlated to the growth of the body (represented by the sitting height) or to the increase in the nasal height. The relative increase in the sitting height considerably exceeds that of the nasal breadth. The height of the nose also shows more rapid growth than the breadth. The latter relation becomes still more marked during postnatal growth. As a consequence, the nasal index decreases from early uterine to adult life. The relative increase in size of both height and breadth of the nose seems to be especially small in the fifth and seventh month of pregnancy, while the most active growth occurs prior to the fifth month. These conditions indicate a certain rhythm of growth, which may be similar to the one established for the body-height after birth. During the entire development the averages of the nasal breadth of negroes are, without exception, greater than in whites. The ranges of variation of this measurement in the two races, however, overlap.

A constant and well-marked racial difference is also found in the relative nasal breadth. As early as the eleventh week fetuses show this difference, which becomes quite marked after the fourth month. The averages in negroes are always greater than the corresponding ones in whites. In both races the averages of this index fall rapidly from the tenth to the fifteenth week—as a matter of fact, almost one-third of the average which it has reached at the end of this period. During the fifth month this relative measurement drops still lower in whites and increases slightly in negroes. Thereafter it increases irregularly, to reach a second maximum

TABLE 6.—*Relative ranges of variation.*

Week.	Nasal breadth.
12th....	32
13th....	48
14th....	46
15th....	31
16th....	29
17th....	22

in both races during the eighth month (see curves in figure 3). Martin (1914) gives figures for the relative nasal breadth of adult negroes, which equal those of negro fetuses of the eleventh week. In the author's adult negro material it does not reach these high figures. In whites the nasal breadth diminishes during growth from about one-third to one-fourth of the interzygomatic breadth. The considerable ranges of variation of the relative nasal breadth lead to the conclusion

TABLE 7.—*Relative increases in size.*

Age.	Nasal breadth.	Nasal height.	Sitting height.
11th week..	17	18	23
12th week..	25	30	30
13th week..	15	20	22
14th week..	17	20	24
15th week..	21	15	17
16th week..	7	12	9
17th week..	9	9	10
18th week..	10	21	11
19th week..	4	5	7
20th week..	6	1	7
22d week...	12	12	8
24th week..	13	9	15
7th month..	3	7	10
8th month..	17	15	16
9th month..	12	6	13
10th month.	-2	9	17

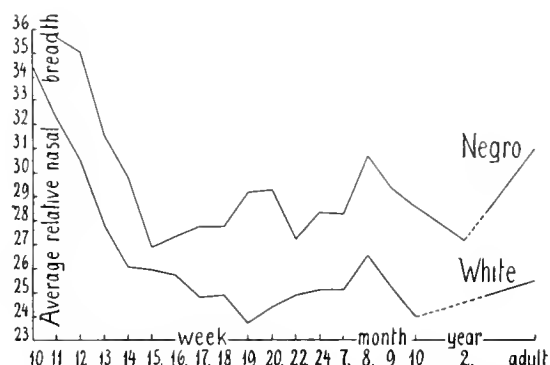


FIGURE 3.—Curves of average relative nasal breadths.

that there is but little correlation between the breadth of the nose and the breadth of the face. Generally speaking, the relative growth of the latter during intra-uterine development exceeds the relative growth of the former. In postnatal life this relation is reversed. For racial diagnosis the relative nasal breadth could be used to greater advantage than the relative nasal height. Very high values for this index constitute an almost certain indication of negro blood in a given case;

TABLE 8.—*Averages and ranges of variation of the nasal index and averages of the upper facial index.*

Age.	Nasal index.						Upper-face index.	
	Whites.			Negroes.			Whites.	Negroes.
	Min.	Av.	Max.	Min.	Av.	Max.	Av.	Av.
10th week.....	106.7	116.8	126.7				43.4	
11th week.....	97.4	117.4	166.7	117.6	128.8	147.1	39.9	40.6
12th week.....	97.9	111.8	150.0	106.4	121.9	137.5	39.1	41.2
13th week.....	80.0	106.7	130.0	120.4	124.9	130.2	38.2	40.2
14th week.....	87.5	104.6	123.1	111.3	129.8	160.8	37.3	37.3
15th week.....	92.5	110.1	123.1	112.5	114.0	116.7	35.3	35.8
16th week.....	87.0	105.3	125.0	112.5	112.5	112.5	36.5	37.8
17th week.....	88.9	104.6	125.0	110.0	112.9	121.1	36.4	38.6
18th week.....	83.3	94.8	105.3	100.0	104.5	109.1	37.5	38.5
19th week.....	83.3	93.3	110.0	104.4	111.3	118.2	37.3	38.8
20th week.....	86.4	98.2	110.0	108.3	113.3	118.2	36.7	40.6
21st, 22d weeks...	80.0	98.3	109.1	85.7	102.0	117.9	37.0	40.0
23d, 24th weeks...	92.9	102.0	107.7	103.6	111.5	115.4	37.3	40.2
7th month.....	86.7	98.2	105.7	93.8	107.7	123.1	38.6	42.8
8th month.....	90.0	100.7	106.7	105.9	120.5	133.3	41.6	43.4
9th month.....	105.6	105.6	105.6	105.3	123.7	133.3	35.8	39.2
10th month.....	80.4	92.6	110.0	99.1	113.2	129.4	40.0	40.2
Children.....				74.3	93.6	120.0		47.0
Adults.....	50.8	60.9	78.0	66.1	77.6	89.8	57.6	58.6

an index below 24 is sure to be found only in whites. The extremes of this index, as found in fetuses, are 20.6 and 40.0.

The Japanese fetus of the eleventh week had a relative nasal breadth of 31.7—*i. e.*, below the average of whites of the same age. The four Filipinos show the following values for this relative measurement: Eleventh week, 36.4; fourteenth week, 29.6; nineteenth week, 31.4; twenty-first week, 25.0. The three Indians: Sixteenth week, 29.6; eighteenth week, 30.6; twentieth week, 27.9. All the Filipinos, as well as Indians, show greater relative nasal breadths than the averages in whites of corresponding ages.

Of the two macacus fetuses of Toldt, the younger had a relative nasal breadth of 37.5, the older one of 36.4. The gorilla fetus of Duckworth (1902), with a sitting height of 71 mm., had a relative nasal breadth of 34.1, and the one described by Deniker (1887), with a sitting height of 136 mm., had a nasal breadth of 38.8. These figures of catarrhine apes are higher than those of corresponding stages of human fetuses, and are situated at the upper end of the range of variation of this measurement in man.

#### NASAL INDEX.

Table 8 (on p. 181) gives the averages and ranges of variation of the nasal index and the averages of the upper facial index. The relative ranges of variation for the nasal index of whites are as shown in table 9. The nasal index has a variability which is at least equal to that of the absolute nasal measurements. This leads to the conclusion that there can exist no close correlation between nasal height and breadth, which constitute the index. Broca (1872) calculated the nasal index in 21 skulls of fetuses from the fourth to the fifth month; the relative range of variation of this series was 44. The relative range of variation of the nasal index in the 25 adult negroes and the 51

TABLE 9.

Week.	Nasal index.
12th....	42
13th....	48
14th....	34
15th....	28
16th....	36
17th....	34

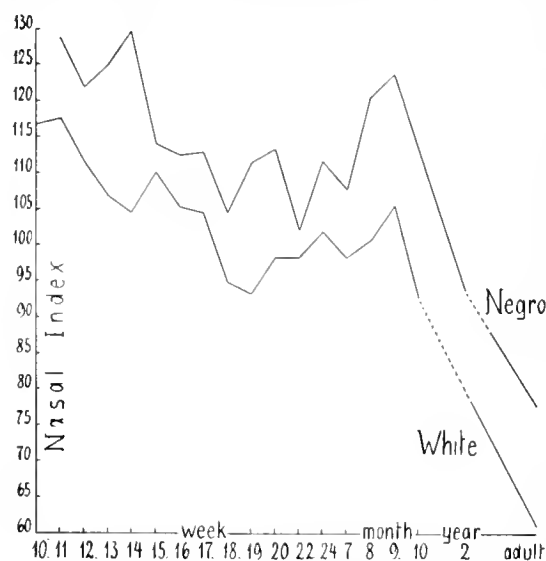


FIGURE 4.—Curves of average nasal indices.

great variability of the nasal index is as well marked in fetuses as in adults, and as pronounced in the fetal nasal skeleton as in the fetal external nose. Broca's conclusion that "la forme de la region nasale est bien plus sujette que la forme générale du crâne aux caprices des variations individuelles" needs only to be changed in reference to the external nose and head to hold good for fetuses. The extremes of the nasal index in fetuses are 80.0 and 166.7.

A racial difference of this index is found during the entire development, the negroes showing always greater averages than the whites (see curves in fig. 4). The greater fluctuation of the curve of growth of the

nasal index of negroes is due to the smaller number of individuals in their age groups, in which occasional extreme variants would change the average to a considerable degree. The racial difference exists even in the youngest stages studied and is almost exclusively the result of the difference in the nasal breadth of the two races. The averages of the nasal index of negroes are always above 100; only in a few individuals was the index lower. In whites, on the other hand, every age group has an average nasal index below 100. With but few exceptions negro fetuses are hyperchamærrhine; white fetuses are frequently chamærrhine and even in some cases only mesorrhine. In both races the index drops during the first five months and increases again during the latter part of fetal development, and to a greater extent in negroes than in whites.

The changes in the nasal index are dependent upon the relation of the relative increases in size of nasal height and nasal breadth. If the relative increase of the former exceeds that of the latter the index will drop; *e. g.*, in the series of whites of the eighteenth week the relative increase of the nasal height was 21 and that of the nasal breadth 10; the index drops, therefore, from 104.6 to 94.7. After birth the nasal index steadily decreases but always shows the racial difference. Only a few races in the adult stage have a nasal index indicating a nasal breadth that is at least equal to the nasal height—a very frequent finding in fetuses; among them may be mentioned Australians, Tasmanians, Bushmen, and certain negro tribes. According to Broca, the nasal index of the bony nose also decreases during intrauterine development, but here the values are much below the corresponding ones of the external nose as a consequence of the great difference between nasal breadth and breadth of the apertura piriformis, the latter being much smaller.<sup>1</sup> Table 10 shows Broca's figures for the nasal index in skulls of white fetuses.

The nasal index stands in correlation with the body-height, the increase of the latter being followed by a decrease of the former during the entire development. Collignon (1887), Houzé (1889), and Pittard (1911) have shown that this correlation exists also in adults, inasmuch as groups of individuals with greater body-height have a smaller average nasal index than those with smaller body-height.

That the form of the nose is not correlated to the form of the face can be seen from a comparison of the nasal index and upper facial index in table 8; the latter changes during fetal growth only to a small degree and its averages in negroes are always greater than those in whites; that is to say, a negro fetus will have a relatively higher face combined with a relatively broader nose than a white fetus.

The Japanese fetus of the eleventh week had a nasal index of 122.6, the four Filipinos 121.2 in the eleventh week, 137.9 in the fourteenth week, 117.4 in the nineteenth week, and 108.3 in the twenty-first week; the three Indians 123.1 in

TABLE 10.—*Nasal indices of fetal skulls.*

Age (months).	Speci- mens.	Nasal index.
2.5 to 3 months..	6	76.80
3 to 4 months..	5	77.68
4 to 5 months..	21	64.88
5 to 6 months..	8	63.01
6 to 8 months..	23	62.25
8 to full term...	24	62.18

<sup>1</sup>In fetuses up to the sixth month the width of the apertura is only little more than half the breadth of the external nose.

the sixteenth week, 110.0 in the eighteenth week, and 120.0 in the twentieth week. All of these figures are above the averages in whites of corresponding ages.

Of the monkey fetuses previously used for comparison, the younger macacus had a nasal index of 75.0, the older one 72.7; the younger gorilla 93.7, and the older one 95.0. These values are lower than those of corresponding stages in man, which is largely a consequence of the greater facial and nasal height in apes.

#### INTEROCULAR BREADTH.

Table 11 is a compilation of the averages and ranges of variation of the absolute and relative interocular breadth and of the relation between nasal breadth and interocular breadth. The relative ranges of variation of the interocular breadth of

TABLE 11.

Age.	Interocular breadth.						Relative interocular breadth.						Lower to upper nasal breadth.					
	Whites.			Negroes.			Whites.			Negroes.			Whites.			Negroes.		
	Min.	Av.	Max.	Min.	Av.	Max.	Min.	Av.	Max.	Min.	Av.	Max.	Min.	Av.	Max.	Min.	Av.	Max.
10th week.....	4.0	4.70	5.2	.....	.....	.....	45.4	48.9	52.6	.....	.....	.....	61.5	70.7	79.2	.....	.....	.....
11th week.....	4.3	5.10	6.0	4.3	4.95	5.7	32.9	42.7	63.2	36.9	40.4	43.8	58.3	76.3	88.9	79.0	89.0	104.2
12th week.....	5.3	6.18	7.0	6.0	6.0	6.0	34.1	38.8	44.7	37.5	40.2	42.9	60.0	78.6	93.5	83.3	87.5	91.7
13th week.....	6.0	7.35	9.0	7.0	7.0	7.0	31.0	36.7	44.4	35.0	36.0	38.0	62.5	76.3	104.8	76.8	86.1	92.9
14th week.....	7.0	8.87	10.5	7.0	8.50	9.0	29.6	35.3	43.8	29.0	32.5	36.0	55.0	74.0	87.5	85.7	91.6	100.0
15th week.....	8.5	10.06	11.0	9.0	9.50	10.0	27.3	32.9	36.7	28.6	31.0	32.3	60.0	79.2	94.4	77.8	86.9	100.0
16th week.....	9.0	10.48	12.0	9.0	9.25	9.5	27.8	31.8	35.5	28.0	28.1	28.1	68.2	81.0	90.9	88.9	97.3	100.0
17th week.....	10.0	11.74	14.0	10.5	11.42	12.3	29.3	31.6	35.3	28.2	30.6	33.2	64.3	78.6	90.9	81.8	90.5	100.0
18th week.....	10.5	12.86	14.0	12.0	12.5	13.0	27.9	31.5	35.1	30.0	30.1	30.2	67.9	79.2	95.2	91.7	92.0	92.3
19th week.....	10.5	13.50	15.0	12.0	12.0	12.0	26.9	30.2	32.6	26.1	28.0	30.0	71.4	78.3	91.7	100.0	104.1	108.3
20th week.....	12.0	13.50	16.0	14.0	14.0	14.0	25.5	29.5	33.3	30.4	31.5	32.6	75.0	83.0	92.3	92.9	92.9	92.9
21st, 22d weeks....	13.0	14.36	17.0	12.0	13.50	15.0	25.0	28.6	33.3	25.5	27.2	29.4	75.0	87.2	96.9	86.7	100.2	110.0
23d, 24th week....	14.0	15.33	17.0	14.5	15.17	16.0	25.5	27.3	31.5	27.8	29.0	31.4	81.3	92.3	100.0	90.6	98.0	103.5
7th month.....	15.0	16.75	21.0	14.0	15.30	16.0	26.3	28.9	35.0	25.4	27.0	29.6	66.7	87.5	100.0	100.0	104.5	107.1
8th month.....	16.0	18.50	20.0	15.0	17.80	20.0	25.8	28.7	31.0	26.2	28.9	30.8	80.0	92.7	106.3	96.3	106.9	121.2
9th month.....	17.0	18.0	19.0	16.0	19.50	23.0	23.0	23.8	24.7	23.2	26.9	30.3	100.0	105.9	111.8	95.0	110.0	125.0
10th month.....	17.0	20.0	23.0	19.0	21.0	23.0	23.5	25.8	30.3	25.3	28.4	32.9	78.3	93.4	115.8	87.0	101.5	110.5
Children.....	.....	.....	.....	22.0	26.1	28.0	.....	.....	.....	24.8	27.6	32.2	.....	.....	.....	82.1	98.9	107.7
Adults.....	26.0	30.1	33.0	30.0	35.2	42.0	17.2	21.8	25.0	20.8	25.6	31.3	103.2	118.0	153.8	95.2	121.7	151.5

whites are as shown in table 12. With an average relative range of variation of 32.6 the interocular breadth seems to be somewhat less variable than the nasal breadth and height. During fetal life the averages of the interocular breadth are smaller in negroes than in whites, with the exception of the twentieth week and the ninth and tenth months. These differences are for the most part, however, so insignificant that no great stress can be placed upon them. The relative interocular breadth is also rather variable, but does not equal the relative nasal breadth in this respect, the latter having an average relative range of variation for whites from the twelfth to the seventeenth week of 36.1, the corresponding figure for the former being only 28.9. The interocular breadth shows, therefore, a closer correlation to the interzygomatic breadth than does the nasal breadth. In both races the relative interocular breadth drops rapidly until the end of the fourth month; thereafter the decrease is only small, especially in

TABLE 12.—Relative ranges of variation.

Week.	Interocular breadth.
12th....	28
13th....	40
14th....	40
15th....	26
16th....	29
17th....	33



negroes. The racial difference in the relative interocular breadth is not constant during intrauterine growth. During the early part of the latter negroes show a relatively shorter distance between the eyes, but later it frequently exceeds this measurement in whites, and at birth and in postnatal life is always greater. (See curves in figure 5.) During the entire growth the relative interocular breadth changes to a great extent; the highest index (63.2) was found in a white fetus of 11 weeks, while the lowest figure (17.2) was observed in an adult white.

The Japanese fetus of the eleventh week had a relative interocular breadth of 41.7; the Filipinos had 45.5 in the eleventh week, 35.2 in the fourteenth week, 27.9 in the nineteenth week, and 26.9 in the twenty-first week; the Indians had 33.3 in the sixteenth week, 30.6 in the eighteenth week and 32.6 in the twentieth week.

Of the few monkey fetuses available for comparison, the relative interocular breadth in the younger macaeus was 37.5 and in the older one 54.5 (unquestionably a very rare exception!); the younger gorilla had for this index 27.3, the older one

24.5. The relative interocular breadth in these apes, with the exception of the older macaeus, was smaller than in corresponding human stages, but much greater than in the adult macaeus and gorilla. Fischer (1903) showed that very young monkey fetuses have a relative interocular breadth which is even greater than the above-mentioned figures and equal that of young human fetuses. The ontogeny of monkeys shows, therefore, a condition

similar to the human one—*i. e.*, a rapid decrease in the relative distance between the eyes in the beginning, but one of greater degree than in man, producing a narrower inter-orbital region in adult apes than in man. (In only a very few adult monkeys does this index reach the lowest extreme of its range of varia-

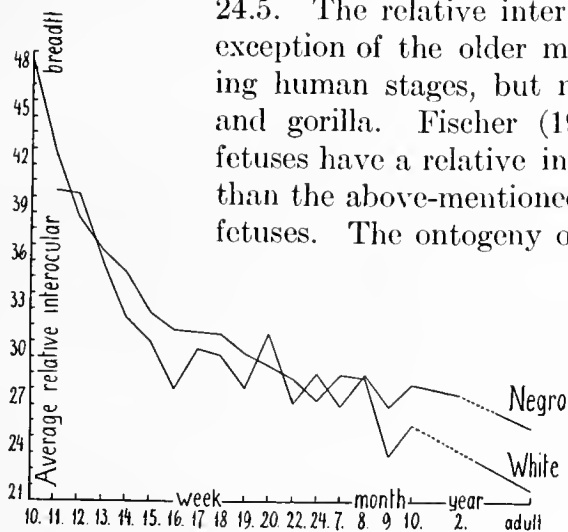


FIGURE 5.—Curves of the average relative interocular breadths.

tion in man.) That in the phylogeny of monkeys there exists a broad inter-orbital region is proved by the fossil skull of *Mesopithecus pentelici*, and Schwalbe (1899) found that the Spy and Neanderthal skulls had a relatively broader interocular breadth than modern man. There can be no doubt, therefore, that in the evolution of primates the eyes have come closer together, narrowing the upper breadth of the nose. This is more marked in apes than in man, and is most pronounced in *Cebus* among the platyrrhines and in *Macacus*, *Cynocephalus*, and *Cercopithecus* among the catarrhines.

The percentage relation between the nasal breadth and the interocular breadth, or between the lower and the upper nasal breadth, shows great variability in the different age groups. The extremes of the entire material were 55.0 and 153.8. It can be stated that the two nasal breadths do not stand in close correlation. During growth this index increases irregularly, the averages in whites ascending from 70 to 118, and in negroes from 89 to 122. Hence in both races the nasal

breadth in the beginning is smaller than the interocular breadth, while later it exceeds the latter. This occurs earlier and becomes more extensive in negroes. The averages of this index are always considerably greater in negroes than in whites, chiefly as a consequence of the difference in the nasal breadth of the two races. (See table 11.)

#### NASAL ANGLES.

In table 13 are given the averages and ranges of variation of the two angles of the nose. The vertical angle, as well as the horizontal, is very variable, probably due in small part to the uncertainty in determining the apex of the nose in fetuses.

TABLE 13.

Age.	Vertical nasal angle.						Horizontal nasal angle.					
	Whites.			Negroes.			Whites.			Negroes.		
	Min.	Av.	Max.	Min.	Av.	Max.	Min.	Av.	Max.	Min.	Av.	Max.
10th week.....	120	125.4	133	.....	.....	.....	90	106.0	116	.....	.....	.....
11th week.....	99	117.4	137	112	120.2	129	85	100.6	120	100	109.0	115
12th week.....	100	116.3	128	122	131.0	140	83	96.8	109	95	103.5	112
13th week.....	90	117.1	140	118	122.3	128	83	98.4	113	85	99.0	107
14th week.....	98	113.8	128	107	112.2	117	82	95.6	110	95	98.7	107
15th week.....	96	110.9	128	108	111.7	116	82	93.9	106	92	103.0	112
16th week.....	97	114.2	130	119	124.0	129	88	96.5	127	106	107.5	109
17th week.....	99	110.0	124	118	123.8	132	80	94.4	114	94	103.0	125
18th week.....	107	114.5	125	112	118.0	124	84	92.8	108	89	102.0	115
19th week.....	100	110.3	125	98	104.0	110	83	89.6	103	88	91.5	95
20th week.....	96	107.8	120	113	115.5	118	85	93.1	110	103	104.5	106
21st, 22d weeks...	98	107.7	118	110	113.2	120	80	88.7	99	86	99.2	107
23d, 24th weeks...	102	106.2	114	10	117.0	132	85	91.5	97	93	102.0	118
7th month.....	92	103.0	115	100	111.7	120	81	90.8	104	98	100.0	102
8th month.....	100	105.7	111	97	101.4	108	85	91.0	97	102	108.0	113
9th month.....	113	115.0	117	92	108.0	116	83	87.0	91	96	100.7	111
10th month.....	86	100.0	108	98	107.6	117	72	87.8	102	90	98.8	108
Children.....	.....	.....	.....	96	112.7	129	.....	.....	.....	90	98.0	107
Adults.....	80	89.5	104	88	100.8	110	49	63.7	76	67	82.4	97

The vertical nasal angle decreases in both races during growth as a consequence of the increase in the nasal depth in relation to the nasal height, and of the moving downward of the apex of the nose. This change during growth is much more marked in the white race, in which the angle drops farther than in the negro and which with few exceptions shows smaller averages. The horizontal nasal angle also decreases in both races during growth. In whites it becomes approximately a right angle as early as the sixth month of intrauterine development and can drop in postnatal life to  $64^{\circ}$ . In negroes the horizontal angle does not fall to  $90^{\circ}$  until late in childhood, and in adults still measures on an average  $82.4^{\circ}$ . The racial difference (*i. e.*, the greater angles in negroes) is more marked in the horizontal angle, the averages of which are without exception smaller in whites.

The two nasal angles express very sensitively the degree of prominence of the nose, as shown, for instance, in their extremes. In whites the extremes of variation of the vertical angle are  $140^{\circ}$  in a fetus of 13 weeks and  $80^{\circ}$  in an adult; while those of the horizontal angle are  $127^{\circ}$  in a fetus of 16 weeks and  $49^{\circ}$  in an adult. How changeable the prominence of the nose may be in individual cases can be seen in

figure 6, which represents the lateral views of the heads of two white fetuses, both in the beginning of the fifth month. The left one shows a very prominent nose and the right one a typical nose for this age. The very prominent hooked nose of a fetus (18 mm. sitting height) described by Gerlach (1884) as a normal variation, is unquestionably pathological. The less prominent snub noses of negro fetuses appear to be still flatter, owing to their deep nasal bridges and the usual existence of protruding eyes in this race. Viewed from the side these specimens show only

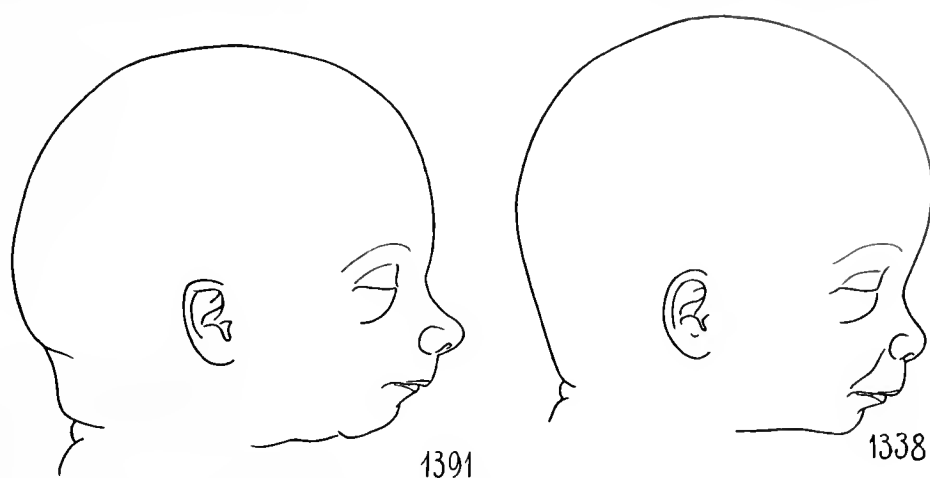


FIGURE 6.—Different degrees of prominence of the nose in white fetuses.

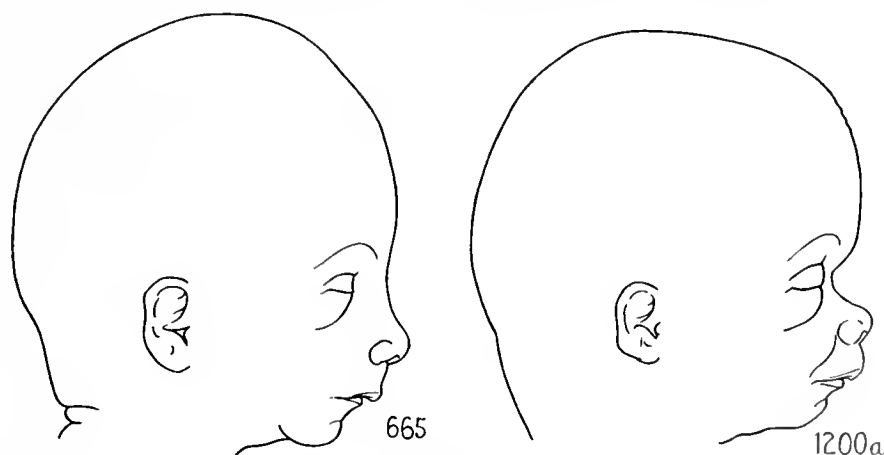


FIGURE 7.—High and deep nasal bridge in white fetuses.

the apical region of the nose projecting beyond the eye and cheek, a condition similar to that found in the adults of some primitive races, as the Eskimos. A deep, concave nasal profile is the rule in negroes and is not infrequent in young white fetuses; the majority of white fetuses, however, show this concavity in only a slight degree. Straight profiles are not rare among older white fetuses, but have been found in only a very few negro ones. In three white fetuses, near full term, there was observed even a slightly convex nasal profile. A "Greek profile," *i. e.*, a high nasal bridge running without break into the forehead, was noted in a few isolated

white fetuses. An example of this type is shown in the left head in figure 7, which was that of a white fetus at the middle of the fifth month. The right head represents the other extreme, viz., a white fetus at the end of the fourth month with a deep nasal bridge and a very prominent glabella.

#### NOSTRILS AND ALÆ NASI.

The smallest breadth of the nasal septum, or the distance between the nostrils, in the third and fourth month is on an average 1.3 to 2.2 mm. It increases during the fifth and sixth month to 2.9 mm. and reaches 4 mm. at birth. In negro fetuses this distance is, as a rule, slightly smaller than in white ones; in adults, however, this breadth averages in whites 6.7 mm. and in negroes 7.4 mm. The variability within the individual age groups is very high. In relation to the nasal breadth the breadth of the nasal septum is much greater in the beginning of fetal development than later on.

The position of the septum as it meets the upper lip is very changeable when compared with the level of the lowest points of the nasal wings where they are attached to the cheeks. The following table shows the percentage frequency with which the different types of attachment are observed:

TABLE 14.

Nasal septum compared with alæ nasi.	Whites.			Negroes.		
	Deeper.	On the same level.	Higher.	Deeper.	On the same level.	Higher.
Third month.....	12	23	65	7	40	53
4th to 6th month....	44	38	18	60	36	4
7th to 10th month...	27	32	41	33	22	45

In both whites and negroes cases of relatively high attachment of the nasal septum are most frequently encountered during the first and last third of intra-uterine life, while from the fourth to the sixth month such specimens are rare and a nasal septum reaching farther down than the alæ nasi forms the rule. These differences in the height of attachment are for the most part rather insignificant, but specimens with a very pronounced difference in this respect were encountered at times. In adult whites the nasal septum was always found to be situated at a lower level than the wings. The opposite relation is a racial peculiarity of the adults of some primitive races—for instance, the Senoi.<sup>1</sup>

In both races the external nares are closed by epidermal plugs up to the end of the fourth month. In rare cases, however, they may be patent at a much earlier date, or may sometimes be found closed in slightly older fetuses. In negro fetuses the nostrils are directed more forward than in whites, a difference which becomes more marked during the later period of pregnancy. Up to the end of the fourth month the nostrils are mostly circular in both races; after that time, in the majority of cases they become elongated, with their longitudinal axes converging forward or

<sup>1</sup>Asymmetries in the position of the alæ nasi could be noted even in fetuses. In No. 1185, for example (white, first part of sixth month), the right wing was situated lower and the left one higher than the nasal septum.

even parallel in whites, while in negroes they usually occupy a transverse position. In one negro fetus (No. 2088) of the beginning of the seventh month, the axes of the nostrils were even diverging forward, a condition resembling that prevailing in many monkeys. In older negro fetuses, and also in some older white ones, the sulcus alaris frequently extends beneath the nostril, thus separating the latter from the upper lip through a fold-like structure (see Nos. 6, 10, 12, plate 1). In younger fetuses of both races the nostrils are frequently found to be some little distance away from the upper lip, but here a sulcus alaris is only indicated and does not extend to the lower surface of the nose. As has been mentioned before, the nostrils of both races are, in the beginning, circular in form, while later on in whites their anterior portions become distended as the result of the increase in depth—*i. e.*, the developing prominence of the nose in this race. In negroes, on the other hand, it is the lateral part of the nostril that becomes distended in consequence of the great increase in the breadth of the nose.

Finally, it may be stated that three white fetuses (Nos. 1823, 1833, and 1903) from the fifth and sixth month showed a distinct sulcus medialis apicis nasi. This sulcus is not of rare occurrence in adult whites, but has never been observed in adult negroes nor in negro fetuses.

#### SUMMARY.

It may be concluded, from the occurrence of the decrease of the relative nasal height, as well as of the relative nasal breadth during intrauterine development, that with advancing fetal age the size of the nose diminishes in relation to the size of the face. The growth of the height of the nose exceeds that of the breadth, which fact is proved by the steadily diminishing nasal index. The relative interocular breadth decreases in the growing fetus, and this to a greater degree than the nasal breadth; therefore, the breadth of the nose manifests a less active growth in its upper part. Besides these general rules, which hold good for both races, this study has shown that the nose of negroes is different from that of whites during the entire fetal period. One of the most marked points of distinction is the nasal breadth, which is greater in negroes, absolutely as well as in relation to the nasal height and to the facial breadth. Further differential characteristics are the blunter appearance of the nose in negroes and the great frequency of a transverse position of the nostrils in older fetuses of that race.

The variability in form as well as in size of the external nose of fetuses is very considerable in all stages.

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## DESCRIPTION OF PLATE.

*Frontal views of fetal heads of whites and negroes showing the development of the external nose.*

No. of head.	Race.	Sitting height (millimeters).	No. in catalogue.
1.....	White....	96	1540
2.....	Negro....	92	1644
3.....	White....	115	831
4.....	Negro....	114	1384
5.....	White....	126	1365
6.....	Negro....	128	1879
7.....	White....	129	1833
8.....	Negro....	132	2086
9.....	White....	207	2049
10.....	Negro....	190	745
11.....	White....	340	2229
12.....	Negro....	303	2161



EXTERNAL NOSE IN WHITES AND NEGROES.





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CONTRIBUTIONS TO EMBRYOLOGY, No. 35.

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MUSCULAR CONTRACTION IN TISSUE-CULTURES.

BY MARGARET REED LEWIS,

*Collaborator in the Department of Embryology, Carnegie Institution of Washington.*

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With two plates and six text figures.

## CONTENTS.

	PAGE.
Method.....	193
Types of muscle.....	194
Smooth muscle from the amnion.....	194
Growth from the amnion in tissue-cultures.....	194
Contraction of the smooth-muscle cells.....	197
Comparison of the growth in tissue-cultures with cells of the normal amnion.....	199
Fixed preparations.....	200
Heart muscle.....	201
Growth from the heart muscle in tissue-cultures.....	202
Contraction of the heart-muscle cell.....	202
Comparison with cells of the normal heart .....	203
Skeletal muscle.....	203
Growth from the skeletal muscle in tissue-cultures.....	201
Contraction of the skeletal-muscle cell.....	205
Other cross-striated muscular tissue.....	205
Discussion.....	207
Conclusion.....	209
Bibliography .....	210
Description of plates .....	212

# MUSCULAR CONTRACTION IN TISSUE-CULTURES.

BY MARGARET REED LEWIS.

The solution of the problem of the structure and behavior of muscular tissue has been frequently undertaken either from the standpoint of detailed and complicated cell architecture or from that of the mechanics of dead material. It is by no means claimed that the observations herein recorded solve this most interesting problem; nevertheless the results show that it is possible to consider the question entirely from the standpoint of living material and at the same time to be able to disregard to a great extent the complicated structure of differentiated muscular tissue. In other words the mechanism to be observed can be reduced to the simple terms of a single slightly differentiated, living cell maintained under observation throughout experimentation.

## METHOD.

Although a new procedure, the tissue-culture method is well known, in view of the fact that when it was first used conclusive results were obtained in two of the important problems of anatomy and physiology—*i. e.*, that the axons grow out from the nerve cells (Harrison, 1910) and that the heart muscle is capable of independent contraction (Burrows, 1912). The method used in the present experiments, while similar in most respects to that of Dr. Harrison and also that of Dr. Burrows, was devised for the study of the cells of the blood, bone marrow, and spleen, in relation to tuberculosis, independent of the work of the above authors. The original solution contained agar. Since it was found (Lewis and Lewis, 1912) that the presence of agar is not necessary for growth of the cells, the Locke-Lewis solution, modified (M. R. Lewis, 1916) according to the species of animal and also according to the osmotic pressure of the tissue to be explanted, has been employed.

Tissues from chick embryos of 4 to 12 days' incubation were used for the cultures. The medium was Locke-Lewis solution (90 c.c. of NaCl 0.9 per cent + KCl 0.042 per cent +  $\text{CaCl}_2$  0.025 per cent +  $\text{NaHCO}_3$  0.02 per cent + 10 c.c. of chicken bouillon + 0.25 per cent dextrose; Lewis and Lewis, 1915). Aseptic conditions were maintained throughout. The embryo was removed from the egg and placed in a petri dish containing 20 c.c. of warm solution. Pieces of the tissues to be explanted were removed, washed through one or more changes of warm medium, and cut up with sharp scissors into pieces about 0.5 mm. in diameter. Each piece was then placed on the center of a cover slip, part of the drop drawn off, and the cover slip sealed on to a vaseline ring around the well of a hollow-ground slide. Cultures thus prepared were kept in an incubator at 39° C. All microscopical observations and experiments were made in a warm box at 39° C.

The cells began to migrate out from the explanted piece within a few hours. At the end of 18 hours a zone of cells, usually only a few cells deep but often wider

than the explanted piece, was formed. Among the cells of this new growth were many mitotic figures (figs. 7, 8, and 9). As the cells migrated out they became spread out more and more closely upon the under surface of the cover slip, so that the edge of the growth was composed of a single layer of large, flat cells. These flat cells, although somewhat distorted in so far as the position of certain cytoplasmic structures are concerned, have an advantage over those obtained by means of sections, in that all the structures of the cell are present and can be observed in their relations throughout the activity of the living cell. The region between these outer cells and the explanted piece may be one or several cells in depth. The cells here, while largely spread out, resemble more nearly the cells of the normal embryo and are very little distorted laterally.

Spontaneously contracting cells usually were found among the less spread-out cells and not among those at the edge of the growth. Cultures of the amnion furnished numerous rhythmically contracting smooth-muscle cells; those of the heart gave rise frequently to sheets of cells contracting in co-ordination with the beat of the explanted piece, and at times to a few isolated cells beating independently; while from the skeletal muscle were obtained muscle buds, muscle-fibers, and myoblasts, each of which were occasionally found undergoing spontaneous contraction.

For a better understanding of the cells in tissue-culture various preparations of other living muscular tissues were made, among which may be mentioned the following: (1) The entire uninjured amnion of a 3, 4 or 5 day chick embryo; (2) a 2 to 3 day chick embryo with beating heart; (3) preparations of teased heart muscle and teased skeletal muscle-fibers of chick embryos; (4) certain microscopical marine copepods whose cross-striated muscle-fibers could be studied while the animal remained alive; (5) the isolated sarcostyles of the insect's wing muscle; (6) thin slices of the muscle-fibers from an adult dog, cat, or turtle. In addition to the above, preparations were fixed and stained in various ways in order to compare the results with those of other investigators.

## TYPES OF MUSCLE.

### SMOOTH MUSCLE FROM THE AMNION.

#### GROWTH FROM THE AMNION IN TISSUE-CULTURES.

The amnion, as is well known, consists of a layer of smooth-muscle cells overlying a layer of epithelial cells, in neither of which have nerve-cells or fibers been satisfactorily demonstrated (fig. 1). The outgrowth from this tissue in cultures did not usually form a membrane composed of the two types of cells, but instead the smooth-muscle cells (sm) and the epithelial cell (E) grew out more or less independently of each other (fig. 9). No nerve-fibers were found in any cultures from the amnion, regardless of the age of the chick. This behavior is quite contrary to that of cultures from certain other muscles (heart, stomach, and intestine) in that a large percentage of cultures of the heart and intestine contain nerve-fibers. The lack of growth of nerve-fibers may be taken as an indication that no ganglion cells resembling those of the heart or intestines were present in the

explanted pieces of amnion. The epithelial cells were readily distinguishable from the smooth-muscle cells, since they became spread out as large, flat, more or less hexagonal cells, frequently united together in the form of a membrane (E), while the muscle cells were either in the form of slender bands or large flat cells (sm) decidedly elongated in the direction along which migration had taken place (fig. 9). In either case the muscle cells were characterized by a peculiar refraction of the cytoplasm due to some substance which took part in its constitution. This phenomenon is exhibited to a certain extent by all the cells in tissue cultures—for instance, where a process of a cell is curled in active movement. All types of muscle cells, however, have a much greater refraction than have other cells. This characteristic increased coincidentally with the maturing of the cell and was especially marked in cells which were undergoing rhythmical contraction. Levi (1916*b*) mentions that the muscle cells from the heart can be distinguished from the mesenchyme cells by a difference in their opacity.

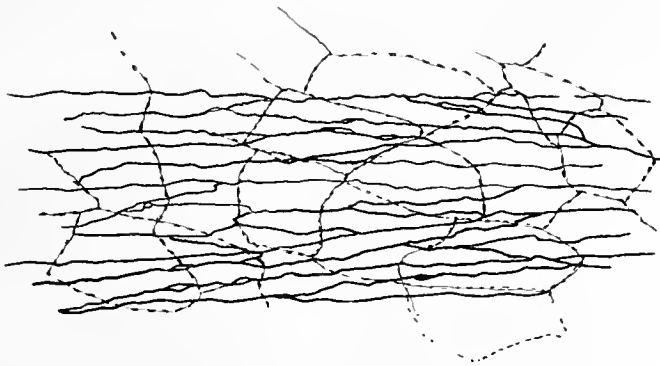


FIG. 1.—Normal amnion from a 6-day chick embryo. Film preparation. Silver nitrate. Heavy lines indicate smooth-muscle cells. Dotted lines show the epithelial cells.

The elongated, band-like, smooth-muscle cells were usually found near the explanted piece; they practically always exhibited rhythmical contraction. The large, flat cells were located mostly along the edge of the growth and seldom contracted rhythmically unless stimulated to do so. The shape and behavior of a cell was determined to a large extent by its position in the growth. A band-like cell one day undergoing rhythmical contraction might the next day, through migration, have become one of the large, thin cells along the edge of the growth.

Many of the large, flat, smooth-muscle cells displayed a marked tension along the cover-slip, so much so that, in some instances, the cytoplasm was drawn into slight folds which frequently extended from one cell to another, through the long axis of adjacent cells, as marked intercellular bridges. Many neighboring cells were joined also by more delicate lateral processes. This living growth, however, is not a syneytium in the usual sense of the term, for even the most pronounced connections between the cells were frequently withdrawn during the migration of a cell away from its neighbors, or by reason of the mitotic division of the cell. It was possible to cause the withdrawal of the intercellular bridges by various experimental procedures; for instance, a minute amount of glycerine introduced into the neighborhood of the growth caused all cell processes to be immediately withdrawn so that the cells became isolated individual cells and remained so

for several hours after the abnormal environment had been removed. This reaction of the cells may account for the failure of certain observers to find connections between the smooth-muscle cells. Certainly the methods of teasing the preparation, described by Schaffer (1899), may account for the fact that isolated or partly teased-out cells in his preparations did not possess intercellular bridges.

No myofibrils were observed in the cells of the living cultures. Except where lines of tension were evident, the cytoplasm appeared as a homogeneous substance in which were embedded granules of different sorts. Very slight disturbances of the cell, however, led to the formation of threads within the cytoplasm. All fixed muscle cells contained fibrils of varying thicknesses located over the surface, where no threads could be distinguished previous to fixation. The appearance of these lines corresponds exactly to what has been described by other observers (Verzar, McGill, Benda, etc.) as myofibrils, although no myofibrils were present in the living cells. Thus the living smooth muscle is characterized by some substance within the cytoplasm which possesses a peculiar refraction, while the dead tissue contains the typical myofibrils. In all probability the myofibrils exist in the living cell in the form of this characteristic material.

That the fibrils are formed by the coagulation of the cytoplasm can be observed directly by watching under the microscope the influence of various substances upon the living cell. For instance, the progressive action of a minute drop of dilute lactic acid placed on the border of the culture drop of medium caused the formation of lines of coagulation, accompanied very shortly by the death of the cell. This occurred first in the outermost cells and later in the thicker cells near the explanted piece. While both the coarse and the fine fibrils appeared in most of the cells upon fixation, the fine fibrils predominated in the more spread-out cells and the coarse fibrils were more evident in the thicker cells. Figure 17 is a careful drawing of a cell after the formation of the fibrils. Before fixation there were no fibrils present in the cytoplasm. The only indications of tension were slightly more refractive regions at the two ends of the elongated cells and a dim line across the nucleus, as though the cytoplasm had been drawn slightly thicker in the long axis. The mitochondria were clearly distinguishable as shiny, slightly wavy filaments. These bright threads did not remain in any one shape but became more or less wavy, occasionally separated into shorter lengths or united together again, and, in short, exhibited activity characteristic of mitochondria in the cells of tissue cultures. This activity demonstrates that there was no structure present in the protoplasm sufficiently dense in nature to interfere with their movement. Upon fixation the protoplasm formed numerous coarse and fine threads, while the mitochondria did not undergo any change. The threads of coagulated protoplasm are quite different in appearance from the mitochondria (fig. 17). The coarse fibrils spread out into finer ones in much the same manner as that depicted by McGill (1907, fig. 23). This appearance is such as to suggest that the fibril was due to coagulation.

Those cells in process of mitosis before explantation completed their division in the cultures. Other cells along the edge of the pieces divided, so that, from the

beginning of growth, mitotic figures were found here and there among the migrating cells. While mitosis of the contracting cells was observed in only one case, it was a frequent occurrence among the large flat cells. The process took place in the same manner as has been described for various cells in tissue cultures (Lewis and Lewis, 1917*c*). During the division of the cell, fibrils were not formed across the cell upon fixation. Just what happens to change the behavior of the contractile tissue at this time is not known, but it is probably involved with the factors at work in the phenomenon of division. The daughter-cells, however, contain fibrils after fixation. Champy (1914), through observations upon fixed cultures alone, found that the dividing cells did not contain myofibrils. From this he advanced the theory that differentiated structures become lost in the growth in cultures due to the rapid mitosis of the cell. This is certainly not true in the cultures of the amnion, for the cells retain their ability not only to contract, but also to form fibrils upon fixation in spite of the fact that mitosis frequently occurs. Epithelial cells in the same cultures did not exhibit the same refraction as did the smooth-muscle cells, nor were fibrils formed in them upon fixation, even when an epithelial cell was side by side with a smooth-muscle cell (fig. 12).

#### CONTRACTION OF THE SMOOTH-MUSCLE CELLS.

Various observers have shown that it is possible for smooth-muscle cells to undergo rhythmical contraction when isolated from the body, either as rings or as strips taken from organs containing smooth muscle (Stiles, 1901; Magnus, 1904; Langley and Magnus, 1905; Roth, 1907; McGill, 1909, etc.). In the experiments of the above authors it was found that various agents stimulated while others inhibited the phenomenon. For general observation, however, Locke's solution was found to be the most favorable medium. The experiments in tissue cultures, while demonstrating unquestionably that it is possible for smooth muscle to undergo rhythmical contraction when entirely separated from the nervous system, have in addition the value of permitting the observer to follow the phenomenon under the microscope and to see just what changes take place in the structure of the individual cell.

The process of contraction of the amnion cells in tissue cultures may be exhibited by several cells, by a single cell, or by only a portion of a cell. There seemed to be present in the cell some active change which caused the protoplasm to be drawn towards a given region. In every case there was a quiet region beyond which no further movement of the protoplasm took place. The result of this current of protoplasm was that the cell became swollen in the region of active change, and usually this area was thrown into folds. The phenomenon (current of protoplasm drawn towards a given region) was exhibited in the same manner many times. The region to which the protoplasm was drawn, and usually piled in

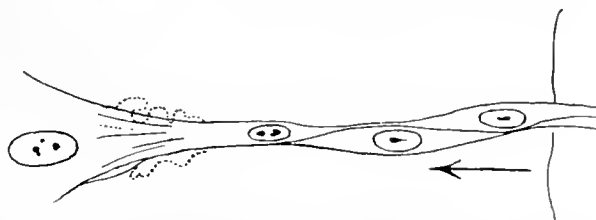


FIG. 2.—A bundle of three contracting cells. The contraction node is indicated by the dotted line. Culture 24 hours old from amnion of 5-day chick embryo. Oc. 6, lens 3 mm.

folds, was a more or less definite one; *i.e.*, roughly speaking, the process was repeated in the same region at a given rate per minute for longer or shorter intervals of time (fig. 2). The area of contraction corresponds with the contraction node described by McGill, as will be shown later. It was not, however, merely an accidental point at which a contraction wave passing through the cell happened to be fixed. The active change then appeared to be neutralized and the protoplasm returned to its former position only to be again drawn towards the same spot after a more or less definite interval of time. In figure 13 a contraction area was located at C in each of three different cells and a rhythmical contraction took place there at a rate of 8 per minute. That the protoplasm actually flowed was demonstrated by the movement (in addition to that of the contractile material) of the nucleus and granules in some cases, and of the granules only in others. The cytoplasm was piled in folds at each C and did not appear as shown in the drawing until after fixation. The contraction node was an expression of the contraction of the cell. There was no wave of an enlarged area sweeping over the cell as is present in the intestine during peristalsis, neither did the behavior appear to be due to a narrower ring passing along the cell. The activity could be described only as that caused by the attraction of protoplasm to a given region.

A number of observers—Remak (1843), Leydig (1849), Schwalbe (1868), Rouget (1881), Marshall (1887), Werner (1894), Schultz (1895)—have described folds in the smooth-muscle cells. These writers claimed that the smooth muscle shortened by a series of zigzag foldings. Such a process does not agree with that displayed by the amnion cells, for in the latter the protoplasm was moved from one region of the cell to another. The cell became larger and was thrown into folds in the region to which the protoplasm was drawn, accompanied by a decrease in the size of the cell in the regions from which the protoplasm was taken. A zigzag shortening might appear to have taken place where the entire muscle cell was drawn into the contracted area, as in the contraction of the total amnion noted below, so that the protoplasm of the whole cell was contained within the enlarged and folded area. This area might then appear as though shortened by means of a folding in of the protoplasm. Kölliker (1849), Heidenhain (1861), Schaffer (1899), Grützner (1904), and Soli (1906) described knob-like thickenings along the surface of the unfixed muscle where the protoplasm, although not folded, is thicker than the remainder of the cell. Such behavior corresponds more nearly with that produced by exposing the smooth-muscle cells in cultures to some abnormal environment rather than to the folded area of normal contraction. In the former case knob-like swellings appear along the surface of the cell and remain for some time.

No change, other than a slight thickening and shortening, was exhibited by either the nucleus or the granules relative to each contraction. Frequently pseudopodia were extended out from the cell or withdrawn into the cell during that period of time in which rhythmical contraction was exhibited. Accompanying the flowing of the cytoplasm toward the region of active change was a swaying or pendular motion which twisted the cell about the long axis. The twisted and bent nuclei described by Van Gebuchten (1889), Heidenhain (1900),



Forster (1904), and McGill (1909) may be due partly to this. In one case, where several cells formed one long strand which was undergoing rhythmical contractions, the pendular movement was so violent that it terminated by one of the cells near the middle of the strand being whirled completely around several times, much as a spool upon two twisted strings whirls about when the latter are loosened and then again pulled taut (fig. 3).

#### COMPARISON OF TISSUE-CULTURE GROWTH WITH CELLS OF THE NORMAL AMNION.

Preparations for a study of an uninjured amnion can be made as follows: The entire blastoderm of a chick (72 to 96 hours incubation) is stretched out on a cover-slip moistened with Locke-Lewis solution. This is then inverted over a hollow ground slide and sealed with vaseline. Another successful method is to place an older embryo in a deeper well and impose the cover slip directly upon the extended amnion. In either case it is important to permit an air space to remain around the embryo, otherwise the contractions will shortly cease. The cessation of activity of the amnion which results when the preparation is entirely covered with medium may, in some measure, be due to the action of carbon dioxide, since Hooker (1912) finds that oxygen is essential for the rhythmicity in vascular muscle. According to Dr. Hooker, if the muscle is exhibiting rhythmicity, this is abolished or depressed by  $\text{CO}_2$ .

Contraction and relaxation of the smooth muscles of the normal amnion resulted in a rocking or swaying motion. This is due to the presence, in various regions throughout the amnion, of a peculiar, star-shaped arrangement of the muscle fibers (fig. 15). Fülleborn (1895) gave a short description of the muscle fibers of the amnion. This author states that the muscles of the amnion of a chick of 5 to 6 days' incubation are short, spindle-shaped cells. During the first half of the development of the embryo these cells grow into long, slender bands. In certain regions these cells are arranged into large and small, star-shaped groups from which the muscles stream out in all directions. Verzar (1907) later published a description of this same star-shaped arrangement, together with an analysis of these muscle centers and their relation to the motion characteristic of the amnion. In the growth from the amnion in tissue cultures this peculiar configuration of muscle fibers was found only among the cells in the immediate vicinity of the explanted piece.

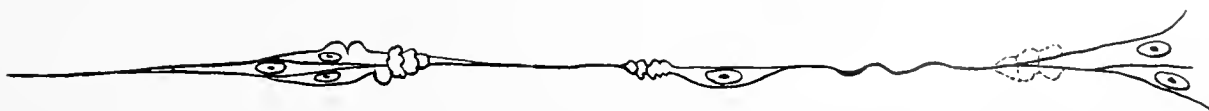


FIG. 3.—A living smooth-muscle cell which was whirled about the muscle strand as the result of the pendular movement. Culture 48 hours old, from amnion of 8-day chick. Oc. 6, lens 3 mm.

Contraction of the normal amnion did not usually involve the entire amnion at any one time. Such activity was exhibited by the cells throughout quite an extensive region. When these cells relaxed the phenomenon was repeated in the same or another area. The cells were drawn together with a swaying motion, so

that the cells, thrown into folds, became much shorter and thicker than they were previously. At times when the action of the amnion was weak, only a small group of cells, or sometimes merely a single cell, underwent rhythmical contraction. In such cases the behavior of the active cells was identical with that exhibited by the cells in tissue cultures. The contraction of the normal amnion, even when very extensive, was usually characterized by a quiet region beyond which no further movement occurred, together with the folds of muscle protoplasm in the area of the active center. This motion was always accompanied by a slight swaying or pendular motion. In other words, while the active region of contraction of the normal amnion was usually occupied by many cells, the phenomenon of contraction, nevertheless, corresponded with that of the cells of tissue cultures.

#### FIXED PREPARATIONS.

When cultures of amnion cells were fixed they became entirely changed in appearance, and they then exhibited a striking resemblance to the results given by other observers for the normal chick amnion. In many of the cells the coagulation took place in such a manner as to duplicate the structure shown in figure 4 by Verzar. In these cells it was difficult to distinguish the mitochondria from the myofibrils, owing to the manner in which the two structures were intermixed, so that it was not surprising that Verzar classed both bodies as myofibrils. However, in cells a little more spread out laterally (fig. 16) it became a simple matter to distinguish between a thread due to the coagulation of the cytoplasm (myofibrils) and the mitochondria, because of the fact that the mitochondria were usually wavy, not straight, the same width throughout, not varying, and ended abruptly instead of branching out into finer threads, as did the fibrils. The centrosome is quite clear in the fixed, spread-out cell, and corresponds with that described by Lenhossek (1899), in that it lies near the nucleus and appears to be double or dumb-bell shaped.

While it must not be forgotten that in these cultures of smooth muscle only embryonic amnion tissue is dealt with, nevertheless, it seems as though a few of the results may be compared with those of adult tissue in such a way as to lead to a better understanding of smooth-muscle tissue in general. For instance, figures like many of those which Miss McGill has shown for other types of smooth muscle can be produced in the culture of the amnion by using the proper method of fixation. A comparison of the coarse and fine fibrils obtained by other investigators with the appearance produced in the smooth-muscle cell by the coagulation of the cytoplasm shows that in all probability the structures are the same. Take, for instance, where Miss McGill (1907*a*) states from her figure 23 that the coarse fibers are bundles of finer fibrils; a study of these amnion cells leads to the conclusion that the process by which this appearance was formed may have been the same as that by which the coarse and fine fibrils of figure 16 and 17 were formed. In other words, the fibrils did not exist as such in the living cells, but some material capable of producing such structures upon coagulation was present.

A number of instances have occurred where the substance along the line of tension extending between cells was coagulated in such a manner as to give the

appearance of coarse fibrils passing from cell to cell, as described by Benda (1902) and McGill (1907*a*, 1909). An interesting picture was obtained by fixing a culture with 10 per cent nitric acid and later staining it (fig. 14). In this case the edge of the cells became coagulated in such a manner as to imitate the appearance described by McGill (1909, fig. 15) as "heavy, elastic fibers" and "the more delicate connective tissue network." However, in this case the delicate connective-tissue network was formed by coagulation of traces of the rapidly withdrawing intercellular bridges and the elastic fibers by the fixation of the curled edges of cells just beginning to retract. Such behavior is not without precedent in the fixation of living material. The work of Levi (1916*a*), Lewis and Robertson (1916), Lewis and Lewis (1917*c*), and Chambers (1917), etc., has demonstrated that the actual threads of the mitotic spindle are not present in the living cell. Cowdry (1914) states that the Nissl substance does not exist in the living nerve cell. Marinesco (1912), Mott (1912), and Lewis and Lewis (1912*b*) claim that neurofibrils are caused by the fixation of the nerve fiber. This by no means infers that myofibrils are artefacts; it merely claims that the substance formed by the cell during differentiation does not necessarily exist as threads in the living cell, but that it assumes this form upon the coagulation of the cytoplasm. At least it can be demonstrated in these smooth-muscle cells of the amnion that threads (myofibrils) appeared upon the fixation of the living cell, where none existed previously.

So far it has proved impossible to fix the area of contraction as it appeared in the living cell, even in cases where the fixing solution was injected through an opening in the ring of vaseline directly upon the contracted cell. As soon as the fixative touched the cell the folds disappeared, although the protoplasm remained thicker and more concentrated in this region. Coincident with this, more or less straight, coarse fibrils were formed (fig. 13). After fixation the appearance of many of the cells was such that it might easily lead to an erroneous conception of the phenomenon of contraction; namely, that it had been caused by a shortening and thickening of the myofibrils in such a manner as to concentrate the protoplasm in this region. When fixed contracted muscle cells were stained (fig. 13), the area of contraction, in which folds had been present before fixation, appeared much the same as that structure termed by McGill (1909) the "contraction node." Concerning this, Miss McGill states:

"During contraction more changes take place in smooth muscle than can be attributed to morphological causes, such as thickening of the myofibrils, etc. At the contraction nodes the staining reaction would indicate that there is a marked chemical reaction taking place also."

This view coincides with that expressed above, that there is a center of active change.

#### HEART-MUSCLE.

Burrows (1912) first described the growth and contraction of the muscular cells arising from pieces of embryonic chick heart explanted in plasma. According to Dr. Burrows, these cells do not contain cross striations, although they undergo rhythmical contraction for 24 to 96 hours. In the new growth there could be

distinguished certain cells that were separated from their neighbors. Each one of these isolated cells contracted with a rhythm quite different from that of the rest of the explanted piece and, moreover, the rate was not necessarily the same in each cell. Lake (1916) described the independent rhythmical contraction of the cells from the heart in plasma, but the results of his observations have added nothing to the facts previously published by Dr. Burrows. Shipley (1916) found that in cultures of the anlage of the chick heart "the embryonic cell which is destined to become heart muscle will differentiate and begin to function even, though removed from its normal environment."

Prior to the experiments of the above investigators, demonstrating the actual contraction of the single heart cell, Gaskell (1882) had shown that in all probability the heart muscle is capable of beating without stimulation from the nervous system. This observer claimed that not only does the beat arise spontaneously in muscular cells, but also that the conduction of the excitation from one part to another takes place through muscular tissue. The action of certain salts upon isolated muscular tissue from the heart has been discussed by Howell (1898), Lingle (1900), and others. The salts shown by these investigators to be necessary for the rhythmical contraction of this tissue are present in the Locke-Lewis solution.

#### GROWTH FROM THE HEART-MUSCLE IN TISSUE-CULTURES.

The growth arising in Lock-Lewis solution from explanted pieces of chick heart (4 to 6 days' incubation) differed only slightly from that described by Burrows (1912). It tended to form a membrane which beat as a whole, so that isolated contracting cells were less frequently seen (fig. 7). The cells were joined together by cytoplasmic bridges extending from all sides. These bridges were not permanent but were formed or withdrawn coincidently with the movement of the cells (figs. 12, 13, and 14, Lewis and Lewis, 1912*a*). During mitosis the cell rounded up and remained attached to its neighbors by only a few delicate, hair-like processes. The plane of division separated the cell into two daughter-cells. Schochaert (1909) found that although the embryonic heart muscle appears to be a syncytium, in reality it is composed of primarily individual cells, since during mitosis the spindle plate is formed, indicating that the heart-muscle cell divides into two cells. There was no evidence of cross-striation in the living cells. Occasionally, upon fixation of the cultures, the cytoplasm became coagulated into fine lines over the surface of the cells. In the few fixed heart-muscle cells studied no typical cross-striations were observed. Levi (1916*b*), however, describes the development of cross-striated fibrils in the growth from the heart in plasma cultures.

#### CONTRACTION OF THE HEART-MUSCLE CELL.

The beating cells were smaller than those of the amnion and remained more nearly oval in shape instead of becoming stretched out into slender bands. Each of the cells observed during contraction exhibited activity throughout the entire cell, never in one portion only. The cytoplasm was drawn towards the center of the cells without being thrown into folds, and resulted in a bellying out of the cell as a whole. This was accompanied by a slight pendular movement. The rate of

the rhythmical contractions was rapid, about 70 to 120 per minute. Owing to the rapid shortening and thickening, together with the slight pendular movement, the phenomenon of contraction exhibited by the few entirely isolated cells observed had the character of a distinct beat as though the single cell constituted in itself a minute force pump rather than an infinitesimal part in such a structure. Figure 4 shows a few of the changes of form through which an isolated contracting cell passed within the period of a few hours. This cell, although exhibiting contractions at the rate of 115 per minute, formed several pseudopodia while under observa-

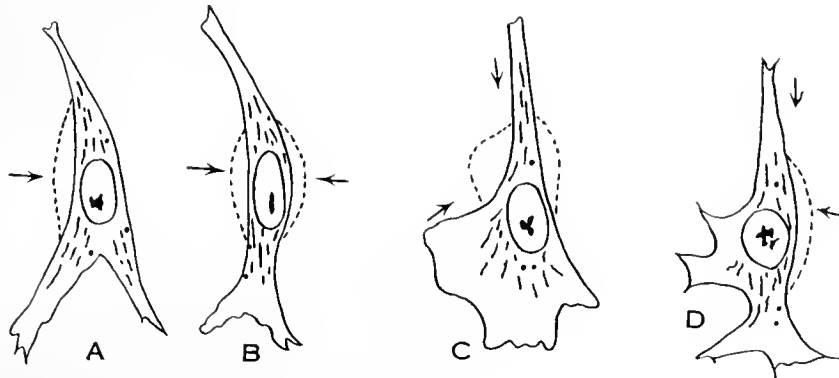


FIG. 4.—Changes in shape exhibited by a heart-muscle cell while undergoing rhythmical contraction. Drawn at intervals of 15 minutes. Rate 115 beats per minute. Culture 48 hours old from heart of 4-day chick embryo. Oc. 4, oil-imm.

tion. The cell was markedly refractive, there were no myofibrils present, and the mitochondria and other granules were neither markedly different from those of any other cell, nor did they undergo any change relative to each contraction of the cell.

#### COMPARISON WITH THE CELLS OF THE NORMAL HEART.

An effort was made to compare the behavior of the heart cells in tissue cultures with those of the normal heart. Preparations of the entire blastoderm (2 to 4 days' incubation) were made in the same manner as that used by Sabin (1917) for the study of the development of the blood-vessels in the living chick embryo. The beating heart was observed with ease, but it proved to be practically impossible to analyze the part played by the individual cell because the coördination of the mass of cells was perfect. The coördination of the beat of the cells of the explanted piece, however, can be disturbed by the addition of calcium to the culture medium. In one such experiment each cell acquired an independent contraction, so that the result was an astonishing dancing of the individual cells without any coördinate beat of the piece as a whole. This peculiar activity was caused by an extremely rapid shortening and thickening, together with a slight pendular movement of each cell.

#### SKELETAL MUSCLE.

Loeb (1899), Garrey (1905), Langley (1908), and Mines (1908) have each discussed the contraction exhibited by isolated skeletal muscle in various media. While the phenomenon continued for only a very brief interval of time in any of the media used by these observers, nevertheless Locke's solution was found to be the most favorable medium for experimental purposes.

The question as to whether cross-striated muscle fiber can undergo regeneration in tissue cultures has been discussed by only a few observers. Sundwall (1912) found almost no proliferation of cross-striated muscle fibers from post partum animals. He states that at the end of 48 hours the cross-striations began to disappear and the terminations of the fibers became more or less globular in form. Even cultures of muscle tissue from 2 cm. embryos showed no growth resembling the original muscle fiber. Congdon (1915) observed that cultures from the limb bud of a 7-day chick embryo show the proliferation of a premuscle cell. The author (1915) gave a short description of the growth in tissue culture of skeletal muscle-fibers which exhibited rhythmical contraction. Levi (1916*b*) described the growth of the heart-muscle tissue and the presence of cross-striated myofibrils in the cells of the new growth. In a few words he states that the growth from skeletal muscle corresponds largely to that from the heart. Lewis and Lewis (1917*b*) gave a detailed account of the structure and behavior of the cross-striated muscle in tissue cultures. These observers obtained the growth of new muscle fibers from the cut ends of the old fibers and, in addition to this, the development of many myoblasts. Certain of the regenerated fibers contained traces of cross-striation. Since then I have obtained as many as 20 to 30 regenerated muscle-fibers in a culture of skeletal muscle from 10-day chick embryos. These new muscle fibers extended out as far as twice, in some cases three times, the width of the explanted piece. Each of the regenerated fibers was cross-striated (fig. 11).

The fact that cross-striations are developed in the muscle cells of tissue-cultures must have an important bearing upon the question as to the nature of the growth in tissue culture. Champy and others contend that the cells "dedifferentiate" into an indifferent type, but if cross-striations can develop it appears as though, under proper conditions, the growth behaves as in regeneration.

#### GROWTH FROM THE SKELETAL MUSCLE IN TISSUE-CULTURES.

The usual growth from an explanted piece of skeletal muscle from a chick embryo (8 to 10 days' incubation) consisted of connective-tissue cells, among which extended numerous muscle-buds, a few isolated muscle-fibers, and many scattered myoblasts (fig. 8). The muscle buds furnish an example of a true syncytium, as the protoplasm of the cells which form these structures is continuous. In fact, they have every appearance of being multinucleated cells. However, when such a muscle sprout was kept under observation it was occasionally found that a cell separated from the multinucleated mass and migrated away as an individual cell, thus demonstrating the probable aggregate nature of the muscle fiber. In addition to this, it has been shown that the protoplasmic ends of two separate muscle-fibers may sometimes fuse together (figs. 3, 4, 8, and 14, Lewis and Lewis, 1917*b*).

The end of the muscle-bud is usually a large protoplasmic syncytium spread out along the cover slip, but the behavior of the muscle fiber (and also of the isolated myoblast) is quite different from that displayed by those from either the amnion or the heart, in that they seldom become spread out laterally into thin

cells, even along the edge of the growth. Fixation causes the coagulation of the cytoplasm along the muscle fiber in the form of a more or less straight fibril. In the ends of the fixed muscle sprouts, however, many fibrils, both coarse and fine, are formed by the coagulation of this material (fig. 10). These fibrils may be straight or curved in various ways, due probably to the amount of contraction of the muscular substance. In some muscle buds the coagulated material resembled the structure termed *primitive myofibril* by Godlewski (1902).

#### CONTRACTION OF THE SKELETAL-MUSCLE CELL.

The skeletal-muscle tissue, whether in the form of a muscle sprout, an isolated fiber, or a myoblast, exhibited contraction as a rather rapid (3 to 120 times per minute, Lewis, 1915) shortening and thickening of the muscular material, with a tendency of the two ends to approximate each other. In the muscle fiber no circular folds were observed along the length of the fiber, neither was there any marked bellying out of the muscular protoplasm at any given region. No folds were found around the myoblasts, but there was a thickening along the middle of the cell (fig. 5). In no case was a pendular movement observed, either by itself or coincident with the shortening and thickening of the muscle cell. It might be stated that the phenomenon of contraction, as shown by the skeletal muscle, differed from that characteristic of the amnion cell and also from that exhibited by the heart cells, in that it was neither a flowing (amnion) nor a beating (heart) movement, but one that more nearly resembled a straight twitch. Spontaneously contracting muscle fibers, and also myoblasts, were frequently found, and in these the rhythmical contractions were exhibited for several hours. At times, however, the activity was induced by some form of stimulation (M. R. Lewis, 1915).

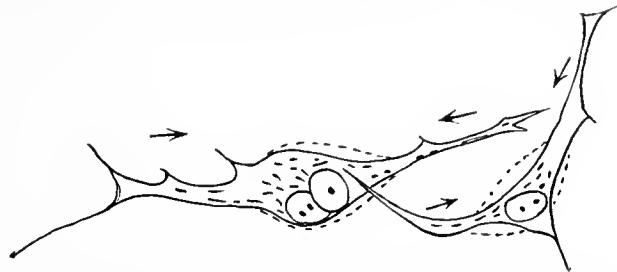


FIG. 5.—Skeletal myoblasts which were undergoing rhythmical contractions. One cell with a rate of 4 per min., the other with a rate of 3 per min. Culture 5 days old from skeletal muscles of a 6-day chick embryo. Oe. 4, oil-imm.

#### OTHER CROSS-STRIATED MUSCULAR TISSUE.

A number of the theories advanced to explain the phenomenon of contraction of muscular tissue have been based largely upon the reactions and appearances of the isolated sarcostyles from the wing muscle of the insect. The isolated sarcostyle proved a fascinating field for experimental investigation, as may be inferred from the numerous observations found in the writings of Krause (1873), Merkel (1872, 1873, 1881), McDougall (1897), and Schäfer (1891, 1912); but it is difficult to understand why certain agents, such as acetic acid following alcohol (Merkel), should have been chosen to obtain results upon which conclusions were to be drawn concerning the action of living tissue. In my observations marked changes were brought about in the appearance of the sarcostyles by almost any change in the medium surrounding them—*i. e.*, dilution, increase in osmotic pressure, increase in the amount of any one of the salts constituting the medium, or the addition of

minute quantities of certain substances, such as acid, alkali, chloroform, nucleic acid, pancreatin, or magnesium sulphate (fig. 6).

Although the addition of weak acid, etc., to the medium did cause the side wall to bulge out, such swelling was not necessarily accompanied by a coincident shortening of the height of the individual sarcomere. Neither was the shortening and thickening of the entire sarcostyle always accompanied by a protrusion of the wall of the sarcomeres. Either the membrane of Krause or the line of Hensen may become extended to a marked degree. Various degrees of extension of the Krause membrane were shown in the same sarcostyle. When the sarcostyle was placed in certain solutions all cross markings disappeared. The details of these experiments will be published in a separate paper, since they throw no light upon the phenomenon of contraction exhibited by muscular tissue in cultures.

Exceedingly thin pieces of the skeletal muscle of the cat, dog, chicken, or turtle, cut with sharp scissors along a line parallel to the length of the fibers, were mounted in a drop of warm Locke-Lewis solution. When such preparations were examined

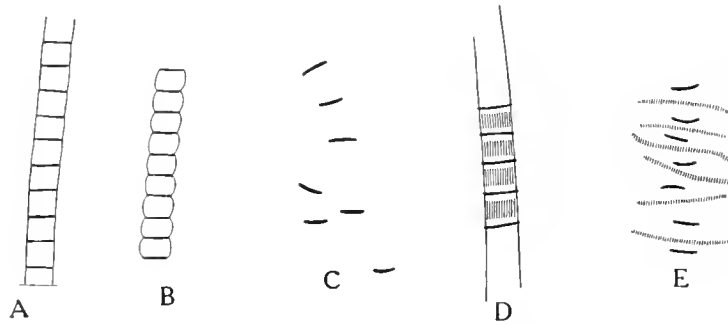


FIG. 6.—Somewhat diagrammatic representations of a few of the many interesting shapes exhibited by the isolated sarcostyles of the house fly. A, normal sarcostyle in fly plasma. B, swollen sarcomeres in slightly acid Locke's solution. C, isolated membranes of Krause floating in Locke's solution saturated with magnesium sulphate. D, fibrillated sarcomeres in Locke's solution containing nucleic acid. E, extended lines of Hensen in diluted white of egg, to which a minute crystal of sodium chloride was added.

under the microscope in the warm box, contraction waves were found occurring in the individual fibers. The contraction was caused by movement of the substance of a given muscle-fiber rather slowly toward a given region, where it became piled up in an area much broader than the width of the muscle fiber. The cross-striations in this region were nearer together than were those in other parts of the fiber. There was no question of the fact that the material of the fiber moved toward this region, for a granule or a foreign body upon such a flowing fiber finally reached and became included within the broader area. The fiber became stretched out at the end from which the protoplasm was flowing, so that the cross-striations in this region became extended far apart. In some cases, while the movement of the protoplasm was taking place, the direction of the current was suddenly reversed, so that the flow occurred in the opposite direction. This lasted only a very short period of time but caused all sorts of distortion of the cross-striations just at the beginning of the broader region (contracted area). In some cases they were drawn out on either side and presented an appearance such as that shown by Schäfer (1912, fig. 286); in others there resulted a tearing of the region adjacent to the contracted area which caused the formation of a homogeneous one resembling that depicted by Meckel. In a number of cases the distortion appeared to be caused by the uneven



pull of the reversed current. Schäfer attributes the homogeneous area to a longitudinal shifting of the fibrils, due to their being unequally pulled upon by the contracted part.

So far as I have been able to determine, either in these preparations of slices of muscle fiber or by a study of the muscular tissue of the living copepod, myofibrils were not exhibited as definite threads in the cross-striated muscle until some change, resulting in the death of the muscle, had occurred. In places where the muscle fiber was not greatly stretched during contraction the reversal of the current did not take place. In such cases the contracted area remained as it was, without either a homogeneous area or an area of distortion between the contracted region and the remainder of the fiber. Such figures have been described by other observers (Flögel, 1872; Hürthle, 1909, etc.). This same phenomenon of contraction waves, with or without the reversed current, were clearly observed in the muscles of the living copepod after experimentation, but it did not resemble the twitch exhibited by the leg muscle of the same animal during normal contraction. In addition to this, the stimulus which brought it about always resulted in the death of the copepod. The piling up of the muscular material in an area which is broader than the muscle fiber, and in which the cross-striations are closer together, is in all probability, as has been shown by Schäfer (1891*a*), an expression of the phenomenon of rigor mortis.

#### DISCUSSION.

Among the muscle cells of tissue-culture growth, whether originating from the amnion, from the heart, or from the skeletal muscles, there are always to be found isolated embryonic muscle cells capable of contraction. The cytoplasm of these cells does not contain any characteristic structure, but is marked by a higher refraction than that of other kinds of cells. The contraction exhibited by each muscle cell is, as above shown, characteristic for the type of tissue from which it arises. In spite of this, however, the actual process is the same for the different types of muscular cells—*i. e.*, there is a point somewhere within the protoplasm of the cell at which some change takes place, drawing the protoplasm towards this region, and resulting in the shortening and thickening of the area involved. A neutralization of the active change then occurs, accompanied by relaxation or a return of the protoplasm to its normal position.

These observations can have little or no bearing on the problem of the causes of muscular contraction without definite physiological experimentation. However, they may throw some further light upon a few of the theories previously advanced. For instance, the change can scarcely be due to the imbibition of water at the point where contraction is exhibited (McDougall, 1897; Meigs, 1908), for in that case there would probably be no currents of protoplasm toward the point at which the change takes place. Since myofibrils can not be demonstrated in these living cells, the activity can not be based upon the increase of fluid within a definite column such as myofibril (Roulé, 1890; Imbert, 1897). The observation of Holmgren (1910) that there is a change of substance from the granules of the cell into the myofibrils is not confirmed, since no such change occurred in the

granules present in these cells, nor were myofibrils observed. Pseudopodia were formed by the cell regardless of the fact that it was undergoing rhythmical contraction; so that while there is a change in the position of the protoplasm during contraction, it is not dependent solely upon those factors which are involved in the formation of pseudopodia (Verworn, 1895).

A few experiments with various fats and soaps were suggested by Quincke's theory of protoplasmic motion. These showed that, while it was possible to cause the formation of pseudopodia (*i. e.*, blebs of clear protoplasm) this change in the position of a given portion of the protoplasm was not accompanied by contraction of the cell. One of these experiments was as follows:

A minute drop of oleic acid was placed in the medium surrounding an amnion smooth-muscle cell. This caused the withdrawal of the intercellular bridges, so that while the cell remained more or less spread out, it appeared as a somewhat elongated, granular cell with knobs of non-granular protoplasm along the sides. From the region of the knobs blebs of clear cytoplasm, which appeared to be much more fluid than that of a normal cell, were rapidly extended and retracted, only to form again in the same or another region. This activity continued for several hours. Frequently, as many as four or five such areas of bleb formation were observed along the surface of a given cell. The protrusion of the blebs, notwithstanding their large size in some cases, was not accompanied by a shortening of the cell, nor was a current of protoplasm induced towards the region from which the bleb was extruded. This activity, while interesting in itself, did not in any manner resemble that of contraction.

In regard to the theory that surface tension plays a part in the phenomenon, the following experiment is rather interesting. Certain inactive amnion smooth-muscle cells were touched with a delicate glass bristle.<sup>1</sup> The cell touched immediately drew together, causing numerous swellings or folds to form on its surface. These resemble the blebs described during mitosis of the cell (Lewis and Lewis, 1917). The folds shortly disappeared, after which rhythmical contraction was initiated and continued to be exhibited for some time. However, this is but another way of stating that mechanical stimulation induced contraction.

Changes in the osmotic pressure of the medium, such as were suggested by the experiments of Beutner (1913), did not sufficiently influence the contraction of the muscle cells in cultures to enable one to draw any definite conclusions. No results were obtained such as would show that the process taking place during contraction of the muscle cells in cultures is comparable to that supposed by Lillie (1901) to take place during the movement of the cilia of certain ctenophores. Rhythmical contractions were exhibited for many hours by the muscle cells in tissue cultures, but it is impossible to estimate what changes may have occurred in the medium immediately surrounding the cell, due to the movements of the cell itself or to chemical interchanges, such as between the air and the medium, waste products and the solution, etc.

From the above observations it might almost be inferred that it is a normal procedure for embryonic muscular tissue to undergo contraction. While in the

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<sup>1</sup> This experiment was performed by Dr. Robert Chambers.

organism this activity becomes controlled by various influences, such as the nervous system, the differentiation of the cells, etc., yet when this tissue is removed from these controlling elements, as in tissue-cultures, the muscle cell again undergoes contraction as a part of its normal life processes. However that may be, the fact remains that in tissue-cultures the muscle cell, whether originating from the amnion, the heart, or the skeletal muscle, may exhibit this property.

#### CONCLUSION.

Contraction, characteristic of the amnion smooth muscle, the heart muscle, and the skeletal muscle, takes place in the growth from these muscles in tissue cultures in Locke-Lewis solution.

The phenomenon of contraction, while characteristic for a given type of tissue, is not dependent upon a complicated muscular structure such as myofibrils.

The mechanism for the study of the phenomenon of contraction may be reduced to a single, slightly differentiated, living cell in Locke-Lewis solution.

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## DESCRIPTION OF PLATES.

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### PLATE I.

- FIG. 7.—Photograph of a fixed preparation of the growth from the heart of a 6-day chick embryo. Culture 48 hours old. Oc. 4, lens 16 mm.
- FIG. 8.—Photograph of a fixed preparation of the growth from the skeletal muscles of a 9-day chick embryo. Culture 48 hours old. Oc. 4, lens, 16 mm.
- FIG. 9.—Photograph of a fixed preparation of the growth from the amnion of a 5-day chick embryo. Culture 48 hours old. Oc. 4, lens 16 mm.
- FIG. 10.—Photograph of the protoplasmic end of a muscle sprout. The contractile substance is coagulated in the form of bundles of threads. Culture 48 hours old, from skeletal muscle of 8-day chick embryo.
- FIG. 11.—Cross-striated muscle fibers in growth from skeletal muscle of a 10-day chick embryo. Culture 5 days old. Fixed by means of Zenker's solution without acid. Stain iron-hematoxylin. Oc. 6, lens 2 mm.

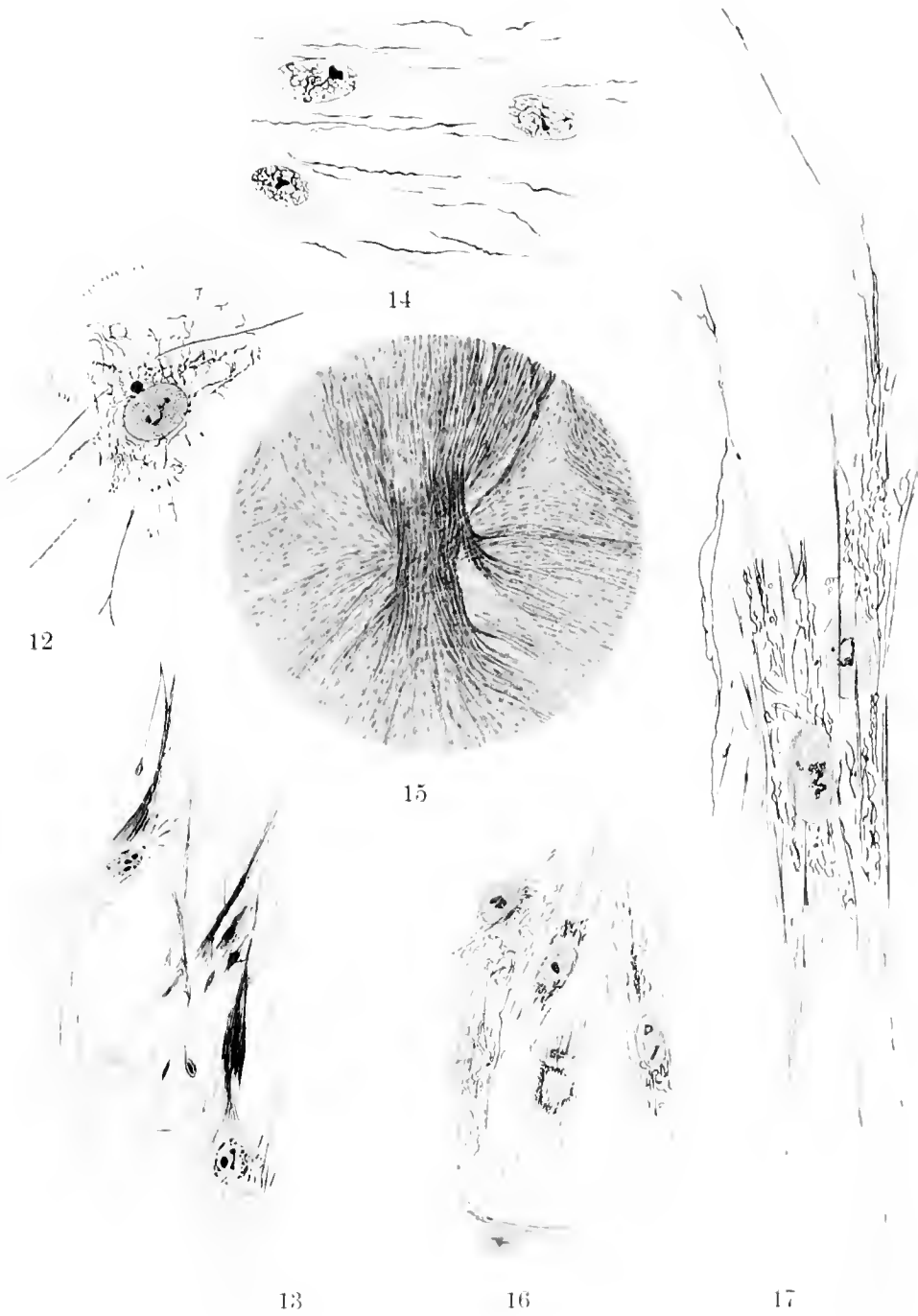
### PLATE II.

- FIG. 12.—An epithelial cell lying side by side with the smooth-muscle cell of fig. 17. It is not elongated, does not contain coagulated contractile substance, and the mitochondria are not long threads.
- FIG. 13.—Three cells observed while undergoing rythmical contraction and later fixed by means of Zenker's solution. C, the contraction node. Culture 25 hours old, from amnion of 8-day chick embryo. Oc. 4, oil-imm.
- FIG. 14.—Smooth muscle from the amnion of an 8-day chick embryo. Culture 48 hours old. Fixed with 10 per cent nitric acid. Stained with Mallory's connective-tissue stain. Curled edges of cell stain as elastic fibers, while the coagulated remains of intercellular bridges appear as a connective-tissue network. Oc. 4, oil-imm.
- FIG. 15.—The star-shaped arrangement of the muscle fibers of the amnion of 5-day chick embryo. Oc. 6, lens 16 mm.
- FIG. 16.—A group of smooth muscle-cells from the amnion of a 4-day chick embryo. Culture 48 hours old. The contractile substance is coagulated into bundles of gray threads. Osmic vapor, iron hematoxylin and eosin. Oc. 6, lens 3 mm.
- FIG. 17.—Spread-out smooth-muscle cell from the edge of the growth. The coagulated contractile material can be distinguished from the elongated mitochondria. Culture 48 hours old from 5-day chick embryo. Oc. 4, oil-immersion lens.











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CONTRIBUTIONS TO EMBRYOLOGY, No. 36.

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STUDIES ON THE ORIGIN OF BLOOD-VESSELS AND OF RED  
BLOOD-CORPUSCLES AS SEEN IN THE LIVING BLASTODERM  
OF CHICKS DURING THE SECOND DAY OF INCUBATION.

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With six plates and one text-figure.

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## CONTENTS.

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	PAGE.
Introduction.....	215
Methods.....	217
Methods of nutrition of early embryos.....	220
Appearances of the exocoelom in the living blastoderm.....	222
Differentiation of angioblasts from mesoderm.....	227
Method of formation of blood-vessels from solid angioblasts.....	231
Origin of blood-islands.....	236
Cycles in the development of the vascular system.....	241
Cycles in cell division.....	252
Origin of the heart and aorta.....	255
Conclusions.....	256
Bibliography.....	257
Explanation of plates.....	259

# STUDIES ON THE ORIGIN OF BLOOD-VESSELS AND OF RED BLOOD-CORPUSCLES AS SEEN IN THE LIVING BLASTODERM OF CHICKS DURING THE SECOND DAY OF INCUBATION.

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BY FLORENCE R. SABIN.

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## INTRODUCTION.

In the study of the origin of endothelium and blood-cells it is obvious that any extension of the method of observing them in living form is an advance. Until recently the tadpole's tail constituted the main subject for the study of growth of the vascular system in a living form. From the time of the discovery by Kölliker that it contains two kinds of capillaries, those carrying blood and those carrying lymph, the tadpole's tail has been studied over and over again in connection with the question of the growth of vessels. The final studies on the living tadpole, made by E. R. Clark (1909, 1918), have demonstrated conclusively that at certain stages both blood-capillaries and lymph-capillaries in the area of the tail grow by the method of sprouting; that is, by the division of and increase in the cytoplasm of the endothelium lining the vessels, and not by the addition of new mesenchymal cells to their walls.

An extension of the study of the vascular system to a living form was then made by Stockard (1915), who watched blood-vessels arise in developing fish embryos. This author showed that the first sign of blood-vessels is the elongation of certain mesenchymal cells into spindle-shaped cells which could be identified as angioblasts. This I believe to be a fundamental point—that the vascular system begins by the differentiation of certain cells which we may term angioblasts, or according to Ranvier, *vasoformative cells*. Stockard then subjected the fish to experimental conditions, by which he made an analysis of the place of origin of red blood-cells. In fish embryos that had been treated by certain chemicals he produced an abnormality consisting of a lack of union between the venous end of the heart and the vitelline veins, so that there was no circulation and consequently no moving of the blood from place to place. From these experiments he concluded that in the fish all of the cells that form red blood-cells are located in the intermediate cell-mass in the posterior part of the embryo, and that none of them arise in the head. In this conclusion his results have been questioned by Reagan (1917) in a study on the same form. Certainly in the chick it can be definitely proved that the endothelium of the vessels gives rise to red blood-cells, and that this process can be seen to take place not only in every part of the area vasculosa, but within the embryo itself, the process having been observed in the aorta in the living chick.

Observations on blood-vessels in living chicks are by no means new; in fact it is obvious that many of the conclusions in the monograph of His, published in

1868, are based upon studies of living chicks immediately after removing them from the shell; while Duval in his atlas, published in 1889, called attention to the advantage of studying the beating of the heart in living chicks in a watch crystal. Indeed this advantage has been quite apparent to the whole group of embryologists using the same material. As a result of the development of different artificial solutions upon which the method of tissue culture depends, there has naturally followed a marked increase in the studies of living embryos of the higher forms; for example, the series of observations made by E. R. and E. L. Clark (1912, 1915) on the superficial lymphatics in chicks held in the shell and kept alive by Rusch solution, and the demonstration by Brachet (1913) that a mammalian egg could be kept alive for at least 48 hours in blood-plasma. These studies, though depending upon the development of the media which are used in tissue-culture, are not specifically tissue-cultures, for by that term is meant the growth of tissues on a coverslip in artificial media, so arranged that the development of their cells can be watched under the microscope.

The first direct application of the method of tissue-culture to the entire blastoderm of the chick—that is, the study of the blastoderm on a coverslip so that its cells could be clearly followed—was made by McWhorter and Whipple in 1912. These investigators followed the technique of Burrows and Carrel, developed from the work of Harrison, using clotted chicken-plasma in which to grow the specimens. In this medium they kept chicks under observation for a considerable period of time—namely, from the stage of about 2 or 3 somites up to the stage of 17 or 18 somites. In my experiments I have followed the method of W. H. and M. R. Lewis, using Locke-Lewis solution instead of the clotted plasma. This technique is much more simple and, I believe, offers many advantages, in spite of the fact that the embryos do not live nearly as long. I have made no attempt to determine the possible duration of life of the specimens in the media, but have watched them on an average of 4 to 5 hours, during which time the maximum number of new somites is 3, with an average of 2. The greater ease of the method renders possible much more extensive observations. My series numbers 266.

With this method of studying the blastoderm of the chick, it is possible to trace the formation of blood-vessels back to their very beginning and to observe them developing by differentiation from mesoderm to a new type of cell, the angioblast. These angioblasts form solid clumps, then bands or cords of cells, which soon unite in a plexus by the well-known method of sprouting. The central part of the substance, either of the isolated clumps or of the plexus of angioblasts, then liquefies, leaving the rim to form the endothelial wall of the vessel. In this liquefaction both cytoplasm and nuclei are involved, and the resulting fluid constitutes the first blood-plasma. Moreover, angioblasts not only give rise to endothelium and blood-plasma, but also produce the red blood-corpuscles. Thus in the chick angioblasts are cells which differentiate from mesoderm and form endothelium, blood-plasma, and red blood-cells.

That angioblasts differentiate in the area vasculosa of the chick has long been known; indeed, the idea is involved in our knowledge of the origin of the so-called

blood-islands dating back to the early embryologists, notably Wolff and Pander. In this study I propose to sharpen the conception of the differentiation of angioblasts and to limit the term *blood-island* to those masses of cells which actually produce hemoglobin and become red blood-corpuscles. It will be shown that though angioblasts, immediately after their differentiation, begin to spread by sprouting, the primitive vessels do not invade the embryo in the manner believed by His, but that there is a progressive differentiation of angioblasts *in situ*, starting in the area opaca of the chick and gradually extending toward the embryo. The heart, most of the dorsal aorta, and even a part of the ventral aorta of the head, can be seen in the living chick to differentiate *in situ*. Thus these studies in the living blastoderm confirm the experiments of Hahn (1909), in which he destroyed the area vasculosa on one side of a very young blastoderm and subsequently found an aorta on that side. These experiments were confirmed later by McWhorter and Miller (1914) and the same point was brought out by Reagan (1915), who isolated the head-fold of a young embryo and found vessels in the isolated segment. It may thus be considered as settled that blood-vessels arise from cells which differentiate from mesoderm, and that these cells (angioblasts) differentiate not only throughout the area vasculosa, but also throughout the body of the embryo itself. The ultimate period at which angioblasts cease to differentiate from mesenchyme must be regarded as still unknown.

#### METHODS.

My early studies were made in the following Locke-Lewis solution: NaCl 0.9, KCl 0.042, CaCl<sub>2</sub> 0.024, NaHCO<sub>3</sub> 0.02, Glucose 0.25—with chicken bouillon.

It is convenient to make stock solutions of the salts four times the desired strength and mix 25 c.c. of each. To this mixture is added the glucose and 20 c.c. of chicken bouillon. This is made according to the method for media and carefully neutralized with sodium bicarbonate. The bouillon has also 0.50 grams of sodium chloride to each 100 c.c. in order not to dilute the Locke solution too much. The mixture of Locke solution and bouillon (Locke-Lewis solution) is then divided into test tubes and heated in an Arnold sterilizer. It may be put through a Bergfelt filter instead, but this has no advantage except to prevent the solution from becoming spoiled during sterilization by the formation of a precipitate, an accident which occasionally happens.

This Locke-Lewis fluid is, of course, a solution in which the exact amount of salts is unknown, owing to the addition of the bouillon; but since a given solution of bouillon can be kept sterile and made to serve for a very large number of experiments, there is a possibility of making certain experiments with a solution which varies only in the known amount of salts added. This solution has been shown by W. H. and M. R. Lewis to be excellent for the growth of tissues of chicks that have been incubated for from 6 to 12 days, but in studying the entire blastoderm in this fluid I noted that in specimens of the latter half of the second day, by which time circulation had been established, nearly all of the hemoglobin was laked out of the corpuscles. Following the studies of Bialaszewicz (1912), who showed that an

unincubated hen-egg contains 1.07 per cent of sodium chloride, and that the amount decreases as the blastoderm develops, I used 1.07, 1.06 and 1.05 per cent.

With these higher percentages of sodium chloride it was evident at once, not only that the hemoglobin was not laked out of the red blood-cells, but that the solution was a more favorable one for all of the cells of the young blastoderm. To determine the best solution for each of the early stages of the chick would necessitate a much longer series of experiments than I have carried through. So far my best results have been with solutions containing 1.06 or 1.05 per cent of sodium chloride in specimens of less than 20 somites. In specimens having between 20 and 30 somites a solution containing 1.07 or 1.06 per cent of sodium chloride will show a considerable amount of crenation of the free red blood-corpuscles, and hence is too strong. It is interesting to note that the young red cells still attached to the islands, or just freed from them, do not tend to crenate as much as the older cells, indicating that there is a slight difference between them. The concentration of the sodium chloride is a very important point for the successful study of the young chick-embryos because all of the cells are very sensitive to it.

The technique of preparing the specimens is as follows: The blastoderm is cut out in a warm box under sterile precautions, leaving a narrow rim around the area opaca; it is then placed in a dish of warm, sterile solution and freed from the yolk and the vitelline membrane, after which it is transferred to a dish of fresh solution and floated on a sterile coverslip. This must be free from grease, as for blood-smears. The coverslip is then placed on a piece of blotting paper and the rim wiped perfectly dry. I mount the blastoderms, for the most part, with the endoderm against the coverslip. This has a twofold advantage: in the first place, the great majority of the vessels develop ventral to the mesoderm and hence are nearer to the endoderm than to the ectoderm; in the second place, the cut edges of the blastoderm always roll dorsalward and hence it is easier to get flat mounts on the ventral surface. Moreover, the endoderm sticks to the glass much more closely than the ectoderm, a matter of great importance in using a thin medium like the Locke-Lewis solution. If the specimen sags away from the glass it interferes very much with the development of the blastoderm. It must therefore be spread out carefully and the edges pulled out to the dry rim of the coverslip, leaving room enough for the rim of vaseline which seals the mount but which must not touch the specimen at any point. If in the mounting of the specimen a little of the blastoderm comes in contact with the vaseline it should be remounted, or else a symmetrical point of the specimen be brought in contact with the vaseline. Otherwise surface tension will greatly distort or even destroy the embryo. The covers are mounted on a hanging-drop slide and the amount of fluid which remains on the blastoderm is sufficient to keep the embryo alive.

Inasmuch as the endoderm of the area opaca is many times as thick as that of the area pellucida, it is clear that there must be a rim of tissue just at the border between the two which will not be in exact contact with the glass. To make this rim as narrow as possible I stretch the specimen on the glass to a considerable extent. If one has a large number of specimens it will become clear that there is a very great



variation in the width of the area pellucida, and since it is only this area that can be seen distinctly in the living specimen, there is considerable variation in the value of different specimens. The stretching of the specimen flattens the embryo somewhat, so that the circulation, if begun, is often not reestablished immediately on the coverslip. In this connection it is interesting to note that the circulation is more often impeded in the left side than in the right, since the heart curves to the right, and hence the left vitelline vein is the longer and becomes compressed across the body of the embryo. In spite of this mechanical disadvantage a good circulation is often (indeed usually) reestablished and maintained for 3 or 4 hours. The slide must, of course, be kept at a temperature of 39° C., either in a stage incubator or in a warm box.

The average life of the specimen is about 5 hours. The heart can often be made to beat, after it has stopped, by a bath in fresh solution, but I have never seen it beat for more than an hour after this procedure. Cell division does not cease when the heart stops, but I have made no tests to determine the actual length of life of cells in these preparations. The most striking sign of the death of the cell in these specimens is that the resting nuclei, which have been practically invisible in the total mounts, become almost as plain as if they had been treated with an acid.

The specimens are fixed by floating the cover-slip on Bouin's fluid of 75 parts of saturated aqueous picric acid, 20 parts of formol and 5 parts of glacial acetic acid. The picric acid is removed by repeated changes of 70 per cent alcohol without the use of any of the lower grades of alcohol or of water. If placed in water the specimens swell and sag away from the glass. I change the alcohol several times the first day, then keep the specimens in 70 per cent alcohol until white, when they are placed in 80 per cent. I have used absolute alcohol, methyl alcohol, and Helly's fluid as fixatives, but with less success.

For stains I have had the best results with hematoxylin alone, or with hematoxylin and a counterstain of eosin (6 parts), orange G (4 parts), and aurantia (1 part). In staining with hematoxylin the specimens must be hurried through water and not left too long in the dilute stain or they will swell, as in water. The young blastoderms react intensely to hematoxylin and nothing in the tissues reacts to an acid stain except the globules of yolk. A blastoderm, therefore, without any counterstain can be analyzed in a total preparation to a considerable extent over the area opaca, while it is seldom possible to focus sharply enough through its thick endoderm in the living specimen. In specimens which have hemoglobin in the cells a counterstain helps to bring out the contrast between angioblasts without any hemoglobin and the true blood-islands. I have used iron hematoxylin, but with less success, for the total mounts. Eosin-azur brings out the basophilic granules of the angioblasts and of the young blood-islands better than hematoxylin, but it is difficult to get good, permanent preparations of total mounts with this stain, since it is impossible to differentiate accurately for a specimen which varies so greatly in thickness. Alum-carmin is also an excellent stain for these specimens.

If it is desired to mount a specimen with the ectoderm against the cover-slip, a method which offers advantages for studying the area opaca, or if the specimen

is to be cut into sections, the blastoderm should be removed from the glass by a single stroke with a Gillette blade under 95 per cent alcohol. If removed in any of the lower grades of alcohol the specimen will shrink and wrinkle, but after the blastoderm is well hardened in 95 per cent alcohol it will remain perfectly flat through the processes of embedding. If a specimen is thus fixed and dehydrated on the glass, cells which have been studied in the living form can be readily identified in the total mounts or even in sections. I clear the mounts in benzene and oil of wintergreen. The only obvious shrinkage is in passing into the oil, then in an occasional specimen the ectoderm and mesoderm may crack, while the endoderm usually stays intact against the glass.

In describing the specimens I shall use the number of somites as an indication of the stage of development. It has become evident, however, that there is a very wide variation in the stage of development of the vascular system with a given number of somites, and moreover, that there is a great difference in the development of the vessels during the interval between the formation of one somite and that of the next. To show the variation of the vessels with reference to the somites, angioblasts may occasionally be seen in a specimen of two somites in the area pellucida, but they are not usually present there until the chick has 4 somites, and never in great numbers until the stage of 5 somites. Again, a blastoderm of 14 somites usually has angioblasts but no vessels in the posterior part of the area pellucida, but I have a specimen of 12 somites in which angioblasts in this area have developed into vessels, the endothelium of which is giving rise to blood-islands.

#### METHODS OF NUTRITION OF EARLY EMBRYOS.

In the study of the blastoderm there is one phenomenon associated with the nutrition of the early embryo which must be clearly recognized, not only on account of its importance physiologically, but because in the living specimen it simulates so closely the formation of blood-vessels. This phenomenon I have called *endodermal blisters*. The early blastoderms receive nourishment in two ways: (1) by wandering cells heavily laden with yolk, which become detached from the thick endoderm of the area opaca and wander throughout the embryo. These cells were described by O. Van der Stricht in 1892 (p. 217) and have been called wandering endodermal cells by Maximow and Danckhoff; (2) by the absorption of fluid substances from the yolk, which occurs in definitely localized areas. If examination be made of any collection of young chick blastoderms that have been stained and mounted with the endoderm against the cover-slip, it will be noted that large numbers of them show hazy lines, especially in the posterior part of the area pellucida (fig. 2, plate 1).

The appearance of the blisters is far more striking in the living form than in fixed specimens; in the former they simulate vessels to such an extent that almost anyone studying living specimens for the first time would regard them as the beginning of the vascular system. As has been said, angioblasts are not found constantly in the area pellucida until the chick has 5 somites; an occasional blastoderm may show the very first angioblasts there at the stage of 2 somites, more often at

the stage of 3 or 4 somites, but in all of these the forerunners of blood-vessels are solid clumps of cells without any lumen and hence not simulating vessels at all, as can be seen on plate 6, figure 27. In such a specimen as the one shown on plate 1, figure 2, where the somites are just beginning, I do not think there is any possibility of the presence of angioblasts in the area pellucida, and these spaces can not be the forerunners of the vascular system. The small dark spots seen in the area pellucida (plate 1, fig. 2) are either globules of free yolk or, in one or two cases, a precipitate of the stain. In the living blastoderms in the early stages these blisters, with their highly refractive contours, make the most striking picture in the entire blastoderm. They appear as isolated spaces of varied size and shape; sometimes there are many small vesicles, or again there are a few large confluent ones resembling multilocular cysts. Their walls are either a fine, sharp, refractive line, in which case the spaces are much distended, or the border appears to be wider, limited by a definite row of cells with an occasional nucleus looking exactly like the nuclei of endothelium, bulging into the lumen. Such blisters are to be seen in section in plate 4, figure 14, from a chick with no somites and of nearly the same stage as the one shown on plate 1, figure 2.

The fluid in these spaces is held in place by irregular threads of endodermal cells which stretch toward the mesoderm; as can be imagined, these threads break readily and hence the spaces may change with great rapidity, even disappearing entirely within a few minutes. In order to find them in sections one must fix a specimen while they are still visible, as was done with that shown on plate 4, figure 14. Moreover, the identification in sections of blisters that had been recorded in the specimen at the time of fixation is possible only in a technique such as I have described, in which the specimen is kept on the cover-slip throughout dehydration. If the blastoderm be removed with care from the glass the blisters are not usually broken.

In the living specimen one can prove definitely that these structures are blebs of endoderm by means of the focusing screw, as their contours can be made to lead over to the thin film of endoderm that covers them. Moreover, if they are not too thick one can focus through them to the mesoderm beneath. An excellent description of this phenomenon is given in the article of McWhorter and Whipple (1912, page 125), except that these authors interpret it as the beginning of blood-vessels; that is, they include these structures with undoubted vessels which they followed in later stages. They describe them as under the ectoderm rather than under the endoderm, and indeed similar blisters do occur under the ectoderm. By my method of studying the specimen with the endoderm against the cover-slip the blisters of the endoderm are much plainer, and I think that they are much more frequent. McWhorter and Whipple also state that they are not to be found after the stage of 10 somites. I find them frequently in the older specimens, for example at the stage of 17 or 18 somites; and in specimens under 5 somites, I would say that they are almost constant.

It will be seen readily that these blisters tend to disappear under the experimental conditions of growth on a cover-slip and that they can not form again.

Thus, to show them best in total blastoderms the specimens should be fixed as soon as they will stick closely on a cover-slip, which is approximately from one-half to one hour. By the time a specimen has been stretched on a cover-slip for four or five hours the blisters have usually all disappeared. If one wishes to convince oneself that these structures are not artefacts, but represent a true physiological process, it is necessary only to cut out a few early blastoderms and examine them immediately in Locke solution under a binocular microscope, which will show the endodermal blisters with great clearness.

The contents of these blisters is nearly always in complete solution, suggesting that they contain a fluid which has been produced by the digestive activity of the endodermal cells. O. Van der Stricht (1899, page 345) described evidences that the cells of the endoderm show a secretory activity, producing the first tissue fluid for the embryo. On the left side of plate 4, figure 14, the blister contains a single wandering cell from the endoderm, while on the right side the space appears empty. The blisters on plate 4, figure 14, are opposite the intermediate zone of the endoderm which marks the border of the area pellucida. The section shows well the three zones of the endoderm, the thin wall of the mid-line, the intermediate zone and the area opaca. In this section the cells of the endoderm over the blisters are full of globules of yolk. In many specimens this endoderm has been stretched until it is a very thin, fine line.

The endodermal blisters, which may be found in any blastoderm of the first two days of incubation and perhaps even later, represent the primitive method by which the embryo is supplied with fluid and nourishment in the early stages, and hence are especially frequent and important in the early stages before the blood-vessels develop. They are areas of absorption of the fluids which bathe the tissues. They represent the absorption by endodermal cells of the fluid of the subgerminal cavity and are of great importance as a demonstration of the method of nutrition of the early stages. They show how the tissue-fluid arises, precedes the blood-plasma, and bathes all of the cells of the growing blastoderm.

All series of sections of chicks throughout the second day of incubation show also the origin of the wandering endodermal cells. The structure of the endoderm of the area opaca is that of a network of cytoplasm with nuclei in the meshes and enormous droplets of yolk in the vacuoles. From this network many individual cells become packed with droplets of yolk and free themselves. The two types of endodermal cells, those fixed in the network and the wandering type, are shown on plate 5, figure 21, and on plate 6, figure 29. In the latter figure the label *en. c.* indicates one that has wandered to a position dorsal to the mesoderm. These endodermal cells may attain enormous size and may wander to any point in the substance of the embryo. Later they may be found within the blood-vessels and may circulate with the blood-cells.

#### APPEARANCES OF THE EXOCÆLOM IN THE LIVING BLASTODERM.

The second phenomenon with which one must become thoroughly familiar in studying the living blastoderm is the varied appearance of the developing exocœlom. The subject is taken up here from the standpoint of the appearance of the

different areas of the coelom, as they can be made out in the developing chick, with reference to the origin of the blood-vessels. At certain stages the exocoelom in the living chick simulates the blood-vessels, although formed by an entirely different process.

The specimen shown in figure 2, plate 1, is in what may be termed the second stage in the development of the mesoderm of the chick. As is well known, in early chick blastoderms of the first day of incubation there is a stage in which the primitive streak has formed, but before the appearance of a head-fold, when the mesoderm is spread out uniformly through the area pellucida and the area opaca except in the pro-amnion. At the stage shown in figure 2, plate 1, it is evident that the mesoderm is more dense opposite the posterior half of the area opaca, and that the outer rim of the area opaca is much mottled. These patches of cells would, of course, be universally termed the *blood-islands of Wolff*, but I wish to question the use of the term *blood-islands* for them. In sections the mottling is seen to be due to isolated patches of mesoderm closely attached to the ectoderm (plate 4, fig. 14). Moreover, it can be noted that though the dorsal margin of the ectoderm has a smooth contour, the ventral border has many filamentous processes pointing toward the endoderm, and that here and there one sees a nucleus in this ragged border of the ectodermal cells which might be interpreted as the beginning of a new group of mesodermal cells from ectoderm.

To the evidence that mesoderm may arise from ectoderm *in situ*, along the edge of the area opaca, may be added that in later stages, after the sinus terminalis has formed, one can still find quite isolated masses of new mesodermal cells lateral to the sinus. Vialleton (1892) called attention to these small masses of cells, attached to the ectoderm, which are to be found entirely outside the area vasculosa in a chick of 11 somites, and interpreted them as the blood-islands of Wolff. During the next year O. Van der Stricht published a note in which he questioned the conclusions of Vialleton, on the ground that these masses of cells had none of the characteristics by which they could be interpreted as blood-islands. This view coincides with my own interpretation of them—*i. e.*, that, like the primitive mesoderm within the area vasculosa, they constitute undifferentiated masses of cells and thus are not to be identified as blood-islands; and that they are destined to form two different tissues, the two layers of cells which form the exocoelom, and the clumps of angioblasts. They are of especial interest as bearing on the question of the origin of the mesoderm.

Thus the evidence seems to me to point toward the view that while the primitive mesoderm of the chick and the mass of mesoderm of the area vasculosa arise at the border of the primitive streak or in the axis of the embryo for the head-mesoderm, there may be a continual, considerable increase in the mesoderm of the area opaca by new cells which are differentiated *in situ* from the ectoderm along the border of the area vasculosa. This is in accord with the view of His and O. Van der Stricht, as opposed to that of v. Kölliker, Hertwig, and Rabl. As was noted by O. Van der Stricht (1895, page 184), no matter how intimate may seem the rela-

tion between the mesodermal masses that develop into angioblasts and the endoderm there is no sign that the endoderm gives rise to angioblasts.

A point which I wish to emphasize is that at the stage shown in figure 2, plate 1, the masses of mesoderm which are so conspicuous in the posterior zone of the blastoderm are as yet without differentiation. They have been called blood-islands, and it will be difficult to change a name with such a long history. I believe, however, that it would be possible to show that these uniform masses of cells are destined, as stated above, to differentiate into two different structures, neither one of which are blood-islands; *i. e.*, two layers of cells placed along the dorsal border of the masses, which are the forerunners of the mesoderm which will border the coelom, and ventral clumps of angioblasts. Moreover, the angioblasts must first make blood-vessels in the chick before any clumps of cells form hemoglobin and become the ancestors of red blood-corpuscles. There is no differentiation in most of the primitive masses of mesoderm before the somites develop, as indicated in figure 14, plate 4, where the dark spots along the ventral border of the mesoderm, seen especially well on the left side, are merely cells in division. In this section the ectoderm is readily distinguishable, for the reason that its cells form a definite row with many nuclei equally spaced. The mesodermal masses have larger, irregularly placed nuclei, and more vacuoles and more droplets of yolk in the cytoplasm.

The next stage in the development of the coelom is shown in plate 1, figure 3, a blastoderm of 2 somites. This is from a chick which was grown for  $3\frac{3}{4}$  hours on a cover-slip. When first taken from the egg it had many endodermal blisters, but they had all disappeared before the specimen was fixed. It showed a primitive streak and a head-fold but no somites. While it was growing on the cover-slip the first cleft, which determines the first and second somites, appeared, and when fixed, the first and second somites were clear. They are still so delicate as to be seen but faintly in the photograph. The latter discloses an interesting point in connection with the primitive streak, namely, a transverse wrinkling of the streak which is a constant phenomenon in the blastoderms of early stages when grown on a cover-slip. It indicates, I think, that the streak is a zone of active growth in length, so that the fixing of the margins of the blastoderm to the cover-slip consequently produces a wrinkling of the tissues at that point. The same wrinkling of the primitive streak is shown in section in figure 14, plate 4. The specimen shown in figure 3, plate 1, was growing vigorously when it was fixed, for the entire endoderm is caught in the phase of nuclear division. There is a defect in a portion of the mesoderm of the posterior part of the area opaca which is not an uncommon abnormality in these chicks that have been grown on a cover-slip. The defect apparent in the mesoderm on the right side of the photograph is not a real one, but a zone where the cells have reacted less to hematoxylin. The specimen was chosen in spite of these defects because it shows so well the formation of the exocoelom in the area opaca.

Throughout the area opaca the mesoderm has formed a network of hollow vesicles so closely packed together that the interspaces are mere lines of cells. In this particular specimen almost all of the primitive mesoderm, as seen in figure 2,

plate 1, has been involved in making these vesicles, and very few of the cells have become angioblasts. About the middle of the area opaca, on either side, are certain denser masses of cells which can be identified as angioblasts, but such a specimen emphasizes the value of not calling the first isolated masses of mesoderm blood-islands or even angioblasts, in spite of the fact that so much of the primitive mesoderm is not always involved in forming the coelom. It will be noticed that these angioblasts lie opposite the septa of the vesicles, as was pointed out by Rückert.

In figure 4, plate 1, is a specimen with 4 somites showing still more differentiation. Here the primitive streak is almost without wrinkles, for the specimen was grown on a cover-slip for only 35 minutes. There are two small endodermal blisters in the margin between the area opaca and the area pellucida at the posterior end of the blastoderm. In the zone of the amnio-cardiac vesicles the coelom is in the form of the large, closely packed vesicles, like those of the area opaca in figure 3, plate 1. This type of vesicle for the coelom is constant over the area of the amnio-cardiac vesicles and over the area opaca.

The middle zone of the area pellucida presents an entirely different appearance, here is to be seen another and an important phase in the development of the exocoelom, a phase with which it is essential to be thoroughly familiar in relation to the study of the vascular system in the living form. It is a stage in which there are no large, definite vesicles, closely packed together like those just described, but rather where there is a delicate network of mesoderm with wide gaps where the mesoderm fails altogether. Such an area is shown in figure 16, plate 4, a photograph of a section passing through the first somite of a chick of 5 somites. About the middle of this section there are two very plain gaps in the mesoderm. The same point is well shown in figure 27, plate 6, from the same area at a later stage, in which there is a network of angioblasts as well as a network of the exocoelom.

It will be seen in the section on plate 4, figure 16, that the delicate network of mesoderm shown in figure 4, plate 1, over the middle part of the area pellucida, is in the form of two layers with an excessively narrow slit between them, quite different from the wide vesicles of the amnio-cardiac region shown in figure 15, plate 4. In fact, the two sections shown in figures 15 and 16, plate 4, are to be compared with the appearance of the mesoderm in the anterior and middle part of the area pellucida in figure 4, plate 1. In the anterior half of the area opaca, on the other hand, two different structures are visible in figure 4, plate 1: the large vesicles of the coelom, and solid bands of cells which tend to lie opposite the edges of the vesicles and which show particularly well on the right side. These bands or cords of cells are angioblasts and, in the specimen itself, are very clear. The posterior part of the area opaca, on the other hand, has dense masses of mesodermal cells which, in the total preparation, seem entirely undifferentiated; that is to say, one can not make out the difference between the coelomic mesoderm and the angioblasts. In a section of the same stage, however, as shown in figure 29, plate 6, it can be seen that the dorsal cells of the mesodermal masses are just forming two definite layers (*mes.*) which are the forerunners of the lining of the exocoelom. This



is the very beginning of the differentiation of the mesoderm of this area into a dorsal or coelomic part and a ventral or vascular layer (*a*).

While it is true that in an occasional section of a specimen of the stage shown in figure 2, plate 1, the very beginning of this differentiation of the coelomic mesoderm can be found in the posterior area, it is not constant or extensive there until the stage of 4 somites. The fact that the formation of the exocoelom is initiated by the arrangement of the mesoderm into two layers of cells was described and illustrated by O. Van der Stricht for the rabbit in 1895. This author stated that the mesodermal cells arrange themselves into two layers, a dorsal layer, the forerunner of the somatopleure, and a ventral layer, the forerunner of the splanchnopleure; and that at the start there are no spaces between these two layers, but gradually there appear isolated clefts which flow together to make the cavity of the coelom. It can thus be made very clear that the coelom forms from the arrangement of the mesoderm into two layers, which then split apart without the destruction of any of the cells. It is this lack of destruction of tissue that I wish to emphasize, since it brings out in strong contrast the fact that the coelom forms by a method entirely different from the methods by which blood-vessels on the one hand, and the cerebro-spinal spaces on the other, are formed.

These three structures, blood-vessels, coelom and the cerebro-spinal spaces, each have a different embryological history and can not be too strongly contrasted. I shall show that the lumen of a blood-vessel forms by the solution or liquefaction of the central part of a solid mass of protoplasm and thus can not be considered as having any relation to tissue-spaces. According to the work of Weed, the arachnoidal spaces form in a mass of mesenchyme. It is interesting to note that in the stages which we are considering in these early chick blastoderms there is no true mesenchyme, but simply mesoderm in layers and angioblasts in solid clumps or masses. Dr. Weed has proved that the arachnoidal spaces come from tissue spaces in a mesenchyme by gradual increase in the size of the mesh of the mesenchyme brought about by the dilatation of existing spaces and by the breaking of some of the strands of the mesenchyme cells. In this process the residual mesenchyme cells ultimately flatten out to make a lining for the cerebro-spinal channels. The same process is to be seen in the formation of the perioticular spaces in the inner ear (Streeter, 1918). On the other hand, the cells which go to make up the coelom line up in the form of two layers, which then split apart without any destruction of tissue. Thus, embryologically, these three structures are entirely different, and are not analogous in any sense. Blood-vessels develop from dense, solid masses of cells; the coelom develops from cells that invaginate to form a mesodermal cavity or, as in the chick, from mesoderm which forms two layers subsequently splitting apart; while the arachnoidal channels come from the spaces of a typical mesenchyme. Blood-vessels are not derived from tissue-spaces; while, on the other hand, the coelom and the arachnoidal spaces both come from tissue-spaces, but by different processes.

In a study of the differentiation of angioblasts in the living chick, one must be able to distinguish them readily from the appearances of the coelom, as seen in figure 4, plate 1, and the point becomes still sharper in the stage of 5 somites



where there are more angioblasts to be seen against the background of the coelom. This is shown on plate 1, figure 5, and plate 2, figure 6, and also in a drawing from a small part of the area pellucida of one of them, figure 27, plate 6. Thus there is a stage when the coelom appears as a plexus of undifferentiated mesoderm dorsal to the plexus of angioblasts.

The plexus of the exocoelom, as shown in figure 27, plate 6, is a delicate network of protoplasm, with nuclei in the nodes and vacuoles and delicate fibrils in the cytoplasm. This network looks not at all unlike a typical mesenchyme except that it is always in definite layers. Here and there are large interspaces which are entirely devoid of mesoderm. Against this delicate network can be seen the massive bands of angioblasts, still connected in places with the parent mesoderm beneath by bands of cells, and with each other by the sprouts so characteristic of angioblasts. The difference between these sharp, definite sprouts (representing the method by which the angioblasts join each other) and the delicate fibrils in the mesh of the mesoderm is well shown in the drawing. Throughout the stages of from 3 to 8 somites these networks must be studied together. The existence of the two networks, that of the coelom and the more ventral plexus of angioblasts, was recognized by His (1868).

It is not my purpose to follow any further in this paper the development of the coelom, because, as the chick grows older, it becomes less and less a source of confusion in connection with the study of the vascular system. Up to the stage of 5 to 7 somites one must be thoroughly familiar with the different appearances of the coelom in the different parts of the area vasculosa, but as time goes on the ventral space occupied by the vascular layer grows wider and the two structures thus cease to be so nearly at the same focussing level; thus the difficulty in keeping them quite distinct practically disappears. In general, however, in studying the living chick there are three different zones of mesoderm in the area pellucida against which the angioblasts must be studied; first, the area of large vesicles which, by fusion into a single cavity, give rise to the amnio-cardiac cavities; second, the fine network of mesoderm of the middle and posterior zones of the area pellucida; and third, the axial zone of the somites and the dense, undifferentiated mesoderm posterior to them. This posterior, axial zone of massive mesoderm is so dense that only an occasional specimen will show the angioblasts with any great clearness against this background. It is, however, an important area for the study of the aorta. Fortunately, the angioblasts of the axial zone opposite the interspaces between the somites are especially clear in the living chick, while the angioblasts of the posterior zone over the undifferentiated mesoderm, opposite which the posterior end of the aorta develops, can also be seen much more clearly in the living specimen in which the cells are dividing.

#### DIFFERENTIATION OF ANGIOBLASTS FROM MESODERM.

At a given stage in the development of the mesoderm of the chick, certain cells differentiate from the mesoderm to become the forerunners of the vascular system. The sequence of this differentiation will be taken up later,

but for the present I will describe the process where it can be seen best in the living chick—that is, in the posterior part of the area pellucida. This will become clear by comparing figures 4 and 5 on plate 1 and all of the figures on plate 2. In this posterior part of the area pellucida the angioblasts do not begin quite as early as in the more anterior part, but they are always more massive. Thus in figure 4, plate 1, there are a few small, isolated clumps of angioblasts in the anterior part of the area pellucida, while in figure 5 of the same plate there is a definite plexus of them in the posterior part. The characteristics of this plexus are best shown on plate 6, figure 27, a drawing of the area included in the square on plate 1, figure 5.

In the living chick the bands of angioblasts are very readily distinguished from the network of the coelom because they are much more refractive than the undifferentiated mesoderm. This refractivity is much increased during the period of cell division, so occasionally when one first takes out a blastoderm the entire network of angioblasts will appear very brilliant; or again, bands of angioblasts, which are at first rather dull, will gradually develop a high refractivity. This refractivity is due to a change in the cytoplasm of cells which precedes the division of nuclei by perhaps an hour or more. It is characteristic also of mesenchyme cells, as has been described by W. H. and M. R. Lewis, but the phenomenon becomes even more striking when one is dealing with such massive structures as the angioblasts. Since all of the angioblasts of an area such as the posterior zone of the area pellucida pass into this refractive phase at the same time, a living specimen in which the angioblasts are about to divide becomes a very brilliant object. Besides a greater refractivity, angioblasts have a denser cytoplasm than the mesoderm. This is due to large numbers of granules in the cytoplasm. These granules are the azur-granules which have been brought out by Maximow as characteristic of young red blood-cells. Due to their presence, the cytoplasm of the angioblasts is very dense in the living form and stains intensely in fixed specimens with all of the basic dyes like hematoxylin. This reaction to dyes is also intensified at the time of division.

Besides this, there is another very important characteristic of the angioblast, namely: the behavior of the cells after division. In watching the living specimen one selects the bands about to divide by the high refractivity of the cytoplasm and then in about an hour the nuclear figures begin to appear, one after another, in quick succession. The only nuclear figure that can be made out in these masses in the living specimen is the metaphase. After the cells have divided the difference between the mesenchyme and the angioblasts is very striking. The mesenchyme cells become excessively irregular, then separate, and the delicate strands of their cytoplasm are reproduced; while the two angioblasts stay together, forming solid masses in which no cell outlines can be discerned in the living specimen. A single, well-differentiated, resting angioblast can be distinguished from mesenchyme. One can not always be sure of a single cell in process of division because both mesenchyme cells and the angioblasts are highly refractive at that time; but two angioblasts can always be recognized by this characteristic formation of apparently syncytial masses. Such a clump of two cells is shown in figure 26, plate 6.

In figure 27, plate 6, as well as in figure 24, plate 5, can be seen another characteristic of angioblasts. Immediately after their differentiation they show a remarkable tendency to send out exceedingly delicate sprouts of cytoplasm toward similar cells. These processes always emanate from a nuclear area, so that when one finds a clump of cells connected with a vessel by slender, solid processes, it is an indication of new cells which have joined the wall. Such sprouts are also shown on the little group of two angioblasts in figure 26, plate 6. By means of this sprouting masses of isolated angioblasts soon form a plexus; thus, even at the stage of 5 somites, at which angioblasts are just differentiating in the area pellucida, there is a very extensive plexus of these bands. In figure 27, plate 6, all of these characteristics are evident. In this specimen the mass of angioblasts showed the protoplasmic changes which precede nuclear division, and the specimen was fixed just as the stage of the metaphase became visible in a few of the nuclei. The bands of angioblasts thus stand out very clearly against the more delicate network of the coelom beneath, as is plain in the photograph of this section (plate 1, figure 5). Here and there the bands of angioblasts themselves still show a little of the character of the original mesoderm; that is to say, they are in the process of differentiating. Again, in many places the bands of angioblasts are still connected by bands of cells with the mesoderm beneath, so that the angioblasts fade into the mesoderm, while in other places they are fully formed and fully connected with each other by characteristic, delicate sprouts, figure 27, plate 6. In such a preparation there can be no doubt as to the relation of the angioblasts to the mesoderm, and this relation is as clear in the living specimen as in the fixed preparation. The origin of the angioblasts from mesoderm was clearly brought out by O. Van der Stricht (1895, page 182) and agrees with the views of Rückert, Mollier, Maximow, Danchakoff, and others.

The characteristics of the solid bands of angioblasts, as seen in the living form, are brought out in figure 20, plate 4, a drawing of a plexus of angioblasts taken from the posterior part of the area pellucida in a chick of 12 somites, while it was growing on the cover-slip. The position is just lateral to the square on plate 2, figure 8, from a slightly younger specimen. In the living blastoderm the bands of angioblasts appear like a complete syncytium. The interspaces are, of course, indistinct when the angioblasts are in focus, because they represent the layer of endoderm above the angioblasts and the layer of mesoderm beneath. There is never any difficulty in distinguishing the definite layer of endoderm by changing the focus, either in the living specimen or in the total preparations. Under certain conditions, however, the endoderm may prove to be a very confusing factor in these studies. For instance, occasionally the endodermal cells may contain so many droplets of yolk that one can not focus through them clearly. Such specimens never clear up and are useless for a study of the blood-vessels beneath. Or, for some unknown reason, the cells of the endoderm may become excessively vacuolated, in which case they are difficult to focus through, both in the living form and in the fixed specimens. Again, the entire endoderm may divide and its cytoplasm in consequence become too opaque to see through. In my early studies I concluded that these specimens had died, but finally saw some of the nuclear figures, and found

that it was only necessary to wait until the phase of division was over, when the endoderm would again clear up. In the specimen from which figure 3, plate 1, is taken, practically every cell of the endoderm has a nuclear figure.

As can be seen in figure 20, plate 4, the nuclei in the living, resting cells can not be made out in these dense bands. The structure has the appearance of a syncytium; there is some network, occasional globules of yolk, and granules in the cytoplasm. The granules I take to be the basophilic granules and the mitochondria. The only time when these bands of angioblasts look as if they were made up of individual cells is when they are passing through the stage of cell division. During this time, both in the living blastoderm and in fixed specimens, each nucleus is surrounded by its own zone of cytoplasm, as is clearly shown in figure 24, plate 5, from a fixed specimen. This chick had 10 somites when taken from the shell, and the heart was just beginning to beat; when fixed it had 11 somites. The heart stopped beating after  $1\frac{1}{2}$  hours, but the embryo lived and was fixed 2 hours later during a cycle of cell division. The blastoderm is shown in figure 8, plate 2, and the area just referred to (fig. 24, plate 5) is within the rectangle drawn on the photograph. The drawing is reversed in position from the photograph. This specimen is one in which the cytoplasm shows the changes of cell division and brings out the fact that after the cytoplasmic period of preparation all the nuclei do not divide at exactly the same moment; hence some of the nuclei are in the prophase, others in the metaphase, and a few in the anaphase. In the living specimen it can be seen that one cell after another divides until all have divided at the end of the cycle.

In figure 24, plate 5, it is clear that the band is still solid; the process of liquefaction (which will be described in the next section) has not yet begun, and after all nuclei have divided the band will again look like syncytium as complete as that shown in figure 20, plate 4. This is more certain in that the specimen from which figure 24, plate 5, is taken has 11 somites and is thus at a stage when the angioblasts of the posterior rim of the area pellucida are in process of growth rather than in the stage of transformation into vessels. The earliest specimen in which I have seen the formation of a lumen in this posterior zone was one of 12 somites; usually, the condition is just beginning at the stage of 13 somites.

It will be noticed in figure 24, plate 5, that along the lower left margin there is a nucleus which has elongated slightly and looks as if it had undergone a slight differentiation into the type of nucleus characteristic of endothelial cells. When the vacuolation takes place there is a real differentiation of the cells along the border into endothelium, both as regards the nuclei and the cytoplasm; that is, the nuclei elongate and the cytoplasm becomes less granular. If one did not know the actual history of a specimen such as is shown in figure 24, plate 5, one might interpret its appearance as evidence that vessels form from angioblasts by a breaking apart of the individual cells of the solid mass, according to the generally accepted idea; but I am convinced from a study of the living form that the essential points in the process of vessel formation are a differentiation of the cells on the margin into endothelium and a liquefaction of part of the original protoplasm of the mass to make blood-plasma, and that this rounding up of the cells of the mass is a part of

the phenomenon of cell division. It can readily be seen that there must be a stage in the differentiation of a single angioblast from mesoderm when it is difficult to determine the fate of a given cell. This point can be seen in almost any chick of about 11 somites in the posterior part of the area pellucida, where there is a very active zone of the differentiation of new angioblasts (figs. 8 and 9, plate 2). Thus, for example, on the right side of figure 24, plate 5, there is a single cell which, while not quite so granular as the main mass of angioblasts, still looks more like an angioblast than like a mesodermal cell. I interpret this cell to be an angioblast about to join the main mass. The same point can be seen in figure 27, plate 6, where there may be some difficulty in determining the actual fate of some of the cells that connect the angioblasts with the mesoderm beneath. As soon as a cell is completely differentiated into an angioblast, and certainly after its first division, there is no difficulty in identification. For example, the small clump of cells ventral to the mesoderm shown in Van der Stricht's figure 2 (1895, page 209), from the blastoderm of a rabbit, can with assurance be identified as a group of angioblasts, just as there is no question of the fate of the clump of two cells shown in my figure 26, plate 6.

#### METHOD OF FORMATION OF BLOOD-VESSELS FROM SOLID ANGIOBLASTS.

The next stage in the development of blood-vessels is the formation of a lumen in these solid bands of angioblasts. In his original description of the development of blood-vessels His (1868) asserts that they are at first solid and then, by some method which he could not make out, acquire a lumen; but that after the lumen is formed the resulting endothelial cells are less granular than the original solid masses. Shortly afterward, in 1871, this process was correctly analyzed by Klein. This work I am quoting from the *Jahresberichte* of 1872, since the original was not accessible. Klein states that certain cells of the deeper layers of the mesoderm become hollow through vacuolization. Through the enlargement of these vacuoles vesicles are formed, the walls of which become endothelium and then, by subsequent division of the endothelium, there develop within the vesicles masses of cells, partly colored yellow with hemoglobin and partly uncolored. Thus in this early work is given perhaps the best description of the actual processes by which vessels can be seen to form in the living chick embryo. I agree with every one of these points except as regards the early stages; *i.e.*, that during the second half of the second day of incubation all of the cells attached to the inner wall of the vessels develop hemoglobin.

All subsequent investigators of blood-vessel development in the chick have described the vacuoles as being seen in the primitive masses of cells which produce blood-vessels; for example, O. Van der Stricht, Rückert, Mollier, Maximow, Dan-chakoff, and Lillie all have called attention to this phenomenon, but the importance of the process first described by Klein is now brought out convincingly in these observations on the living form. If one watches such a band of angioblasts as that seen in figure 20, plate 4, vacuoles will begin to appear in the solid mass, often just under the edge, but many times directly in the center. It is a very striking process in the living chick and I endeavored to obtain drawings of it, but the changes in

form are so rapid that it is very difficult to record them well. By the time a tracing is made with a camera lucida the form is not quite the same and yet the changes can be easily followed with the eye, being not nearly as rapid as the changes occurring in the endodermal blisters. Angioblasts may be completely transformed into vessels during the time that the blastoderms are growing on the cover-slip—that is to say, in 3 or 4 hours—and the process is extensive in one hour.

In watching a living specimen it can be readily seen that the vacuoles increase in size; many of them flow together and ultimately one may watch the entire center disappear from such a band as is shown in figure 20, plate 4. In figure 25, plate 5, from a chick of 13 somites, can be seen an early stage of the process of the solution of the center of a band of angioblasts. This figure is taken from the angioblasts of the posterior part of the area pellucida. Here and there are large vacuoles just under the edges, while the center of the mass is intact. It will be noticed that some of the vacuoles are against the nuclei, a very common occurrence. I consider it important as showing that the vacuolation or liquefaction is a real intracellular process. The usual conception of the formation of a blood-vessel out of angioblasts is that the mass breaks up by the separation of the individual cells in the center of the mass, while the cells on the edge flatten out to form an endothelial border. Instead, there appears to be no flattening out of the border cells, but rather vacuoles, which occur just under the borders, leave a rim of cytoplasm while the center of the mass disappears. There is a real differentiation of the cells thus left along the edges of the vessel, because they no longer look just like the original angioblasts. Their nuclei elongate slightly and their cytoplasm becomes less granular, so that in the living specimen endothelium comes to resemble ground glass (figs. 18 and 19, plate 4). These endothelial cells, however, retain the power of reproducing the more granular type of cytoplasm, which they do in giving rise to blood-islands. During this process of liquefaction at the center of these angioblastic masses, the vessel formed is almost never any larger than the original mass, indicating that the absorption of the fluid present in the surrounding tissue is but slight.

A later stage in the process is shown in figure 23, plate 5, from a blastoderm of the same stage. In this specimen the center of the mass is disappearing and the vacuolation is much more extensive. Here the edges of the vacuoles are more ragged and little shreds of tissue can be seen in them. On the left border of the mass is to be seen a chain of angioblasts in single file. In this chain the same process is going on between two nuclei, giving the clearest picture of it in a single cell. The same point is shown in the upper right sprout of figure 25, plate 5. There is one place where the process of vacuolization can be always found within single cells—namely, at certain stages over the amnio-cardiac vesicles. Here, as will be described later, angioblasts always form in long, slender bands (fig. 7, plate 2) and in these bands one can always find the liquefaction of chains of single angioblasts. I am quite sure, therefore, that the lumen of a vessel is made within the cytoplasm of a cell and not entirely or even mainly by the separation of cells. It can be readily realized that one often sees the separation of individual cells in such a process.

During the phase of division the cells of the bands always look as if they were about to separate into individual cells, as suggested in figure 24, plate 5, but if such a specimen be watched until it passes into a resting stage the mass will again take on the appearance of a syncytium. When a single nucleus is caught in the stage of division, like the one in figure 25, plate 5, it looks also as if the cell were to become separated from the mass.

There is another phenomenon very characteristic of these developing bands of angioblasts which suggests the idea of the breaking apart of the cells to form a vessel, *i. e.*, cell death. Often while the vacuolation is going on, or just before it begins in a mass, occasional cells stand out by virtue of certain special characteristics. This is true also of the blood-islands which develop subsequently. These characteristics are, first, that the cytoplasm becomes perfectly clear except for a few irregular masses, a phenomenon very familiar in certain cells in blood-smears; second, the nuclei show a sharp contour which is unusual in a living cell. In the living specimen the dead cells stain with a dilute solution of neutral red, while the living cells do not react at all to the dye. In fixed specimens they show pycnotic or fragmented nuclei characteristic of dead cells.

A still later stage in the process of liquefaction of angioblasts, by which the lumen of vessels is formed, is shown in figure 28, plate 6, a section from a chick of 11 somites. Here the process is in the edge of the area opaca since all of the angioblasts of the posterior part of the area pellucida are still solid at this stage. In this section the cytoplasm just above the label *L* has almost liquefied, but in the center of the vessel there is still a little clump of cytoplasm labeled *A*, showing a pycnotic nucleus, an indication that there are some degenerative processes going on in the protoplasm. Between the labels *A* and *L* in the same vessel the endothelial border itself is very ragged, showing also some degeneration of cytoplasm.

In certain areas, especially in chicks a little older (for example, during the fourth day of incubation), there are numerous small, completely isolated vesicles, filled with free blood-cells, which look just as if they had become separated from an originally solid mass. Such tiny vesicles are always to be found dorsal to the amnio-cardiac vesicles, opposite the delicate ventral bands of this area which are shown in figure 7, plate 2. This specimen has these small vesicles which are filled with dead cells, as shown by their pycnotic nuclei. In other specimens the vesicles are filled with normal red cells, and I believe are formed, like the rest of the blood-vessels, by a partial solution of the center of the mass and fill up subsequently by division of the cells of their walls—though there might well be some separating of individual cells in the transformation of the solid center of the angioblasts into the lumen of a vessel. However, in all of the masses of angioblasts which I have seen transformed into a vessel in the living specimen, there has been a considerable amount of the liquefaction of cytoplasm.

Just as cell division progresses in cycles, so this process of liquefaction progresses by stages, and if one finds it in a single band of angioblasts all of the bands in that area will show the same. In general one can see the process, in any blastoderm of 6 or 7 somites, taking place in the area pellucida opposite the venous end



of the heart, in the zone in which the vitelline veins are developing. In a chick of 8 or 9 somites it can almost always be found going on in the lower end of the aorta, opposite the undifferentiated mesoderm and just posterior to the somites; while chicks with from 12 to 14 somites will nearly always show the process in the posterior zone of the area pellucida. This zone is the best in which to observe it in the living form.

From these observations it appears to be clear that the lumen of a vessel is produced by the liquefaction of the central mass of angioblasts to make blood-plasma, and that while whole cells are destroyed and some of the original cells may separate from the original mass, the essential process is intracellular and the formation of the blood plasma by liquefaction is one of its important effects. This fact is brought out most strikingly when the lumen of a vessel is formed in chains of single angioblasts and where there can be no question of the lumen developing in potential clefts between cells, since it is within the body of a single cell. The same fact is actually shown by noting that many of the vesicles begin against the nuclei in the syncytial masses. Thus the first blood-plasma is the result of the destruction of large masses of angioblasts by a true cytolysis, and one of the important functions of these primitive masses of angioblasts is the formation of plasma. Since the tissue fluid is formed before the vessels begin, the endothelium, from the beginning, is a membrane which separates two different fluids, plasma and tissue fluid.

As has been said, the process of liquefaction of protoplasm is shown in section in figure 28, plate 6, which is from a vessel just under the edge of the area opaca of a chick of 11 somites. In this section the center of the larger mass has almost disappeared, while the endothelial border is seen full of tiny vacuoles. In the upper part of the section is a blood-island. The lower vessel, on the other hand, shows again the liquefaction of the angioblasts beginning along the edge of the main mass. The endoderm in this section is also interesting, showing both the syncytial network of the cytoplasm and some of the wandering endodermal cells which are becoming free from the network.

The idea that the lumen of a vessel can be considered as intracellular is not new. It was first expressed by Stricker (1866), who conceived the idea from studying the tiny sprouts to be made out along the capillaries in the tail of the living tadpole. Concerning it, he says:

“Wenn ich sage, die Wände der Capillargefässe sind Protoplasma, dann muss ich wohl selbst zugeben, dass sie aus Zellen bestehen; nichts destoweniger liegt ein tiefer und durchgreifender Unterschied zwischen dem, was uns an den Larven klar und unwiderleglich vor Augen tritt, und zwischen dem, was in neuester Zeit aus der Silbermethode deducirt wurde. Nach dieser Deduction sollen die Capillaren aus Zellen zusammengefügt sein, und das Blut in jenen mithin intercellulär fließen; nach dem was sich an der Larve ergibt, ist ein Capillarrohr Protoplasma in Röhrenform, Protoplasma, welches im Innern aushöhlt ist, und wo das Blut gleichsam intracellulär fließt” (page 7).

It was then brought out in a series of interesting papers published in the early seventies. As has been said, it was most clearly and correctly presented by Klein in 1871, and later was expressed by Balfour (1873), Schaefer (1874), Ranvier (1875),



Leboucq (1876), and Wissozky (1877). Ranvier studied the formation of blood-vessels in the omentum of the guinea-pig and in the blastoderm of the chick, and described the vessels as coming from vasoformative cells. He states:

“C'est généralement dans les points nodaux que se produisent les premières cavités vasculaires et les îlots sanguins. Les premières cavités vasculaires sont d'abord des creux remplis de liquide qui s'agrandissent et s'allongent pour canaliser les branches du réseau. Les noyaux et le protoplasma refoulés à la périphérie constituent les premiers éléments de la paroi du vaisseau. Ces éléments, agissant à la manière des cellules glandulaires, sécrètent un liquide, premier plasma du sang, qui distend peu à peu les branches du réseau pour leur donner le diamètre considérable dont nous avons déjà parlé. Les îlots sanguins se forment aux dépens de certaines cellules des cordons vasculaires primitifs qui sont mises en liberté dans leur intérieur au moment de leur canalisation. Ces cellules, relativement peu nombreuses, sont sphériques et contiennent d'abord un seul noyau (page 640).”

With this description I agree, except in considering that the process by which plasma is formed is one of liquefaction of protoplasm rather than a process analogous to secretion. Schaefer studied the formation of capillaries in the skin of a newborn rat and described certain vasoformative cells with vacuoles within the cytoplasm in which developed new, disc-like, adult red corpuscles. The latter point is, of course, entirely out of harmony with our present ideas concerning the origin of red cells; it is more in harmony with our ideas of the destruction of red corpuscles than of their origin.

The work of Wissozky is very interesting. This author began with the study of the reaction of hemoglobin-bearing cells to eosin seen in drops of fresh blood. From this he went on to making flat preparations of the embryonic membranes at the edge of the placenta of the rabbit, fixed the membrane *in toto*, brushed off the epithelium and stained the whole with hematoxylin and eosin. He made similar preparations from the allantois of the chick and the rabbit, and described the formation of single angioblasts, which he called *hematoblasts*, described how they formed a network with numerous processes which he interpreted as indicating amœboid activity, and, finally how vacuoles formed in these solid bands. Thus he says:

“An irgend einer Stelle des soliden haematoblastischen Stranges erscheint zuerst ein durchsichtiger farbloser Streifen, welcher gewöhnlich bogenförmig ist; dieser Streifen erweitert sich ferner, nimmt die Gestalt eines Halbmonder an, seine Enden nähern sich mehr und mehr, um endlich zusammen zu fließen. Auf diese Weise entstehen in dem Protoplasma der haematoblastischen Stränge die beschriebenen Lücken, in welchen die embryonalen Blutkörperchen liegen, umgehen von durchsichtigen, farblosen Ringen.”

With his description he gives a beautiful figure of this vacuolation going on in bands of single angioblasts, very like my figure 25, plate 5.

All of the characteristics of angioblasts are beautifully shown in two figures of Maximow (1909, plate xviii, figs. 1 and 8). These masses of cells (the first taken from the area opaca of a rabbit embryo and the second from the forerunner of the endocardium of the heart of a rabbit) show the azurophile cytoplasm and the tendency to form synectial masses. This is especially true in his figure of the forerunners of the endocardium. In the first figure are shown the irregular processes of

the cells which I have found depended so much upon the concentration of the surrounding fluid. The angioblast in the second figure shows the beginning of the vacuolation by which the solid masses are converted into vessels.

#### ORIGIN OF BLOOD-ISLANDS.

The angioblasts not only give rise to endothelium and to the blood-plasma, but produce red blood-cells or, strictly speaking, erythroblasts. In the two figures from specimen 174 (figs. 23, plate 5, and fig. 26, plate 6) is shown the striking contrast between a vessel in which the central mass is going to liquefy entirely and an adjacent vessel in which some of the original mass is going to form a blood-island. These vessels are just along the border of the dense mesoderm of the posterior zone in a chick of 14 somites. Figure 11, plate 3, is a photograph of this blastoderm, and both drawings are taken from within the square on the photograph. The angioblasts in question are thus going to form a part of the lower end of the aorta. The aorta follows the lateral line of the somites and below the last somite curves outward, following the lateral edge of the dense, axial mesoderm. Outside this zone in this particular specimen, there is a zone of blood-islands in the vessels, and mesial to this area masses of angioblasts are becoming vessels, while the masses on the border between the two areas show both processes. In figure 23, plate 5, the central mass is delicate and the vacuolation very extensive, and from observations on the living one would expect that the lumen would be complete in a short time. In the other drawing, on the other hand, a part of the cytoplasm has become much more dense than the original angioblasts. This more deeply staining mass would appear slightly tinged with yellow in the living specimen, due to the presence of hemoglobin in the cells. In other words, it has become a blood-island. I have watched the formation of such a blood-island from the stage of solid angioblasts, as shown in figure 20, plate 4, so that I know it is not to be interpreted as a new outgrowth from endothelium. The blood-island in figure 26, plate 6, is still attached to the wall by guy ropes of the original angioblasts, as well as by a solid base. In this manner exceedingly large and irregular blood-islands form. Such islands are not different in their behavior from those which arise along the walls of empty vessels from a division of the endothelial cells.

In figure 26, plate 6, there is also another process to be seen, namely, that of differentiation of new angioblasts. To the right of the main vessel is a clump of two angioblasts which have not as yet joined the neighboring vessels nor begun to form a lumen of their own. These two cells were a little farther along the edge of the vessel than is shown in the drawing; they were shifted slightly in the drawing in order to make them come into the same field. Since these cells are going to join the end of the aorta, they are evidence of the fact that the aorta differentiates *in situ*.

It is now necessary to describe how blood-islands form in vessels in which there has been a complete solution of the central cytoplasm, without any of the original angioblasts remaining except those that make an endothelial border for the vessel. In watching the living specimen the change in the appearance of the vessels after the lumina have formed is very striking. While they are in the form of solid angio-

blasts, it is the angioblasts that make the striking feature of a specimen, as shown in figure 20, plate 4. After the lumen has formed it is the exact reverse, for then the interspaces are conspicuous and simulate vessels. This, I think, will be clear from a careful examination of the photograph shown in figure 11, plate 3. In this figure there are pale rings in the area pellucida, showing especially well at about the middle of the area. These rings are the interspaces and the dull network between them are the vessels. The six sharply outlined spots near the undifferentiated mesoderm, posterior to the somites, are defects in the mesoderm similar to those seen in figure 9. In figure 11 the blood-islands can be clearly seen in the gray bands, that is, in the lumina of the vessels.

In the living specimen the endothelium of a fully formed vessel has the appearance of ground glass, as represented in figures 18 and 19, plate 4. The contrast between the two stages, first, the interspaces between angioblasts, and second, the interspaces between blood-vessels, is brought out by comparing figures 20 and 18, plate 4, both of which are taken from living specimens and represent the actual appearance as nearly as possible. The confusion in regard to determining the interspaces from the lumen of the vessels (which is inevitable to one looking at such a specimen for the first time) at a stage before there is any circulation is entirely eliminated after the blood-cells move in response to the beat of the heart. In fixed preparations the chance of confusion is not great.

In an area in which many new blood-islands are forming, one can often find unicellular blood-islands, two of which are shown in figure 18, plate 4. Again, such an island is shown in a section in figure 21, plate 5. One specimen which was growing on the cover-slip had so many of these unicellular islands that there appeared to be almost a duplication of the endothelium. In such specimens, fixed just during the phase of cell division, I find the nuclear spindles placed perpendicular to the wall of the vessel, so that the inner cell which is going to make the blood-island projects directly into the lumen of the vessel at the very start. On the other hand, the nuclear spindles usually lie in the plane of the endothelial wall, as can be seen in any specimen in which the endothelium is dividing to increase the lining of the vessels.

These small unicellular blood-islands develop a granular cytoplasm, as can be made out in figure 21, plate 5. Thus, from the evidence of the living specimen, the small masses of cells, even the unicellular ones, are properly called blood-islands, except for the fact that, strictly speaking, they are not islands at all, being always attached to the walls of vessels. Not only are the islands yellow, but the cells are uniformly yellow with hemoglobin, showing that all of them become erythroblasts. This was pointed out by O. Van der Stricht (1892) in a study of the development of the blood in the chick. He says (page 216):

*"Dès le premier stade, toutes les cellules sanguines présentent un aspect particulier. Leur protoplasma est d'un jaune foncé plus compact que celui des éléments voisins. Chargées donc d'une quantité d'hémoglobine plus ou moins considérable, elles ont, dès leur origine, les caractères du corpuscule rouge."*

The young islands, as seen in figure 18, plate 4, have a smooth contour, even a very definite border. Neither in the living nor in the fixed specimen can one see any cell outlines within the mass. Nevertheless each nucleus has its own zone of cytoplasm which closes in about it when the cells divide. Just as all of the angioblasts divide at the same time (fig. 24, plate 5), so all of the cells in all of the islands of a given area become highly refractive at the same time. This period of high refractivity lasts about an hour or more, and then the observer sees one nucleus after another pass into the metaphase with the chromosomes on the spindle. This is shown in the small drawing (fig. 18); the specimen was fixed just as the record was made on the drawing of the division of the nuclei, and the nuclei were found in the stained specimen in the metaphase. A photograph of this specimen is shown in figure 12, plate 3. Throughout their early stages the contours of the blood-islands are all round and smooth, except for a very interesting phenomenon which is often to be observed, namely, that delicate sprouts of cytoplasm, exactly like those by which the original angioblasts fuse to make a plexus, are put out from the islands. These sprouts creep along the inner wall of the vessels and attach themselves like guy ropes to the wall. They show a very marked tendency to join other neighboring blood-islands, and I have often seen sprouts from two islands (like the small island on the left of figure 18, plate 4, and the nearby unicellular island) join by sprouts which meet half way between the masses. In this way compound islands are formed, cells filling in along the guy ropes until the whole mass becomes a single island. At other times these new sprouts simply seem to serve as extra guy ropes by which the islands are more firmly anchored to the wall. In the living chick the presence of a circulation in a given area does not interfere with the development of the blood-islands, though it is clear that the venous zone of the area vasculosa is less active in the production of new islands than the more posterior arterial zone when the circulation is later established. I have not noticed that a specimen showing a very marked tendency to form islands is one in which there is vigorous circulation in the area in question. The large compound islands often completely shut off the circulation in a given channel, and in the living specimen I have observed that blood-cells which have been circulating get caught by the bridges crossing the lumen and become incorporated into a growing island.

This property of the blood-islands to send out the guy ropes by which they become anchored in many places to the neighboring wall is duplicated by the Kupffer cells in the liver. In sections of the liver of a rabbit that has received repeated doses of trypan-blue one can get an exact duplication of the picture of numerous unicellular blood-islands seen in the young blastoderm of the chick. In examining sections of the liver which have been stained with carmine or any nuclear dye after repeated doses of trypan-blue, one will often find the nucleus of the parent endothelial cell behind a Kupffer cell exactly as that shown in figure 21, plate 5, in the unicellular island labeled *B. i.*, from a chick of 17 somites. Thus it seems to me clear that Kupffer cells do not make an endothelial lining of the capillaries of the liver but constitute another generation of cells from the endothelial wall of these capillaries, exactly like the blood-islands in the embryo chick; and that

they are anchored out into the lumen of the capillaries by numerous guy ropes of cytoplasm. Instead of developing hemoglobin like the analogous cells of the embryo, they develop phagocytic powers to a high degree.

These sprouts of cytoplasm, proceeding from both the angioblasts and the cells of the blood-islands within the lumen of vessels, are referred to many times in the literature as evidence of amœboid activity. In reality, both types of cells have but a slight power of movement from place to place, and the sprouts represent a rather remarkable degree of attraction of similar cells. By means of these processes isolated masses of angioblasts are brought into a plexus, compound blood-islands are formed, and blood-islands are, as it were, more securely anchored to the inner wall of a vessel.

In figure 18, plate 4, a certain number of erythroblasts are seen in the lumina of the vessels. These cells, which have become free from the islands, continue to divide, even while they are circulating. These circulating cells make it quite easy to determine the lumen of a vessel in the living chick, and, after studying the blastoderms in which the circulation has been established, one will never have any difficulty in identifying the lumen. In the specimen shown in figure 12, plate 3, blood was being pumped into the area of the omphalomesenteric arteries, which are just beginning to be indicated in the figure, but there was no movement of the blood in the area represented in figure 18, plate 4. In figure 19, plate 4, is shown a later stage in the formation of blood-islands. This was also drawn while the cells were in the phase of division, and hence each cell in the mass stands out individually. In the fixed specimen the nuclei of more than half of the cells are in the prophase of division. In the resting stage of these older islands the cells are no more definite in the center of the mass than is shown in figure 18, plate 4. The border of the islands, however, now displays the contours of the individual cells instead of the sharp, smooth contour of figure 18. If islands, such as the one shown in figure 19, plate 4, are watched in the living specimen one will see the cells (one after another) free themselves from the edges. This process is surprisingly slow. I have seen it take  $1\frac{1}{2}$  hours for a single cell to become separated from an island.

These preparations give a good chance to test out the idea as to whether the primitive mesamœboid cells are really amœboid at all. The freeing of an individual cell from an island of course involves power on the part of the individual cells to move, but as seen in the living form this movement is very slow and not associated with much, if any, change in the shape of the cell. Moreover, a cell that has just become free from an island, provided there is no current fluid by which it can be carried away, will stay close to the island where it was formed, and one has to watch closely to detect the slight changes in its contour. This is very different from the rapid changes characteristic of the white corpuscles. I am of the opinion that the marked, blunt processes which are found in specimens of erythroblasts of young chick embryos are associated with a reaction to the concentration of the fluid in which they are placed, because these processes can be much increased by simply increasing the concentration of the sodium chloride in the solution. I therefore conclude that the young erythroblasts are sensitive to osmotic pressure, and that

the younger and older cells of a given specimen vary in their reaction to a given concentration of salt; but that the erythroblasts as a whole are characterized by their very slight power of amœboid movement. Their contours change slightly, as seen in a living specimen, but the cells themselves move from place to place exceedingly slowly when not swept along in a current of fluid. They are elastic but not very motile.

In regard to the breaking up of the blood-islands, the only process which I have actually seen in the living chick is the slow freeing of individual cells from the edge of the mass, but so many fixed specimens look as if islands had been caught just as all the cells were going to break apart at once, that I can readily believe this does actually take place.

We have thus described the processes by which two different structures develop out of the primitive mesoderm, the cœlom on the one hand and the blood-vessels on the other. We have shown that blood-vessels arise from cells called angioblasts, which differentiate from mesoderm and produce endothelium, blood-plasma, and red blood-corpuscles. We have emphasized the importance of the destruction of cells in the production of the first blood-plasma and have shown that this plasma is different in origin from the tissue-fluid. Moreover, it has become clear that it is inadvisable to identify as blood-islands the primitive masses of mesoderm which are to give rise to both of these structures, for the reason that they must first split into cells which will form two layers for the cœlom and those which will develop a different type of cytoplasm and form clumps of cells, the forerunners of vessels. Besides this, these original cells are not all the forerunners of blood-cells, but rather are masses which are to be further differentiated into those which form endothelium, with the potentiality of producing cells which can themselves make hemoglobin and those that become blood-islands. Since one can now distinguish all these types of cells, a more restricted terminology would seem to be of value. That is to say, the original masses of cells in the blastoderm, long known as blood-islands, we might call by the general term *primitive mesoderm*, and distinguish three types of cells, *i. e.*, (1) angioblasts; (2) the cells forming blood-islands (cells anchored to the endothelial lining of vessels, which develop hemoglobin in their mass and which are derived either directly from angioblasts or from endothelium); and (3) primitive erythroblasts or cells which have become free from the islands but which go on dividing actively within the lumen of the vessels.

In his studies on living fish embryos Stockard (1915) did not find evidence that endothelium can produce blood-cells in that form. He says (p. 229):

"There are numerous descriptions and illustrations of the origin of blood-cells from the vessel linings in the literature of the last twenty-five years, since Schmidt in 1892 described the transformation of individual endothelial cells into white and red blood-corpuscles. Yet again, I believe that the really skeptical reader will not be at all convinced that such a thing really ever takes place, from the evidence presented in the literature, certainly not from any of the illustrations that have been made of this process. No real vascular endothelial cell has ever been actually observed to metamorphose into a blood-cell, or to divide off another cell which forms a blood-cell, and until such a direct observation is forthcoming one can only question the accuracy of the interpretation of the various observations up to now recorded."

It is this exact observation that has now been made in the blastoderm of a chick by the application of the method of tissue-culture. One can actually see the division of an endothelial cell into two daughter-cells, one of which remains in the wall of the vessel as a part of its endothelial lining, while the other protrudes into the lumen and becomes a unicellular island, the forerunner of a mass of erythroblasts. That endothelium gives rise to erythroblasts may therefore be accepted as proved in the case of the chick. The studies herein recorded do not include any observations of the origin of the white blood-cells, since there are no cells in the chick of the second day incubation that can be identified as the forerunners of the white cells, all of the cells in the islands and free in the vessels having hemoglobin.

In 1892 Schmidt described the origin of both red and white blood-cells from the endothelium of the vessels of the liver and spleen in human embryos. He described localized areas of mitosis in the endothelium of the capillaries of the human liver during development and interpreted them correctly as giving rise to clumps of blood-cells. These he interpreted as both red and white cells. In his own words he concludes (p. 220):

"In der embryonalen Leber findet eine mit der Gefässentwicklung im Zusammenhang stehende Neubildung weisser und rother Blutkörperchen statt. Die ersteren werden von den Endothelien der Capillaren durch karyokinetische Theilung producirt und pflanzen sich selbst durch Mitose weiter fort. Die rothen entstehen aus den farblosen durch Auftreten von Haemoglobin im Protoplasma und besitzen ebenfalls die Fähigkeit äquivalenter Theilung durch Mitose."

Since that time many authors have given evidences of the origin of blood-cells from endothelium, both in birds and in mammals, as will be described later in connection with the origin of blood-cells from the endothelium of the aorta.

#### CYCLES IN THE DEVELOPMENT OF THE VASCULAR SYSTEM.

It will be interesting to take up what I shall call the *cycles* in the development of the vascular system, and which I shall subsequently show are due to successive cycles in cell division. This subject is illustrated in a series of photographs, plates 1 to 3. At the stage shown in figure 2, plate 1, when there is no head-fold, the mesoderm is almost undifferentiated. In the area pellucida there are two zones of mesoderm, an axial, dense mass, and a lateral, less dense zone. In the lateral part there is little indication of the zones which will ultimately divide into three parts; an anterior zone of the amino-cardiac vesicles, a middle zone which will ultimately lie opposite the venous end of the heart, and in which the vitelline veins will develop, and a posterior zone in which the omphalo-mesenteric arteries will appear. Over the area opaca the mesoderm is dense, and over the posterior area, especially, it is much mottled. It has already been brought out that the mass of mesoderm at this stage is but slightly differentiated into the cells that go to make up the exocoelom and those that become angioblasts. In the figure of a slightly older stage given by Rückert (1906, fig. 880, p. 1210) can be seen the differentiation between angioblasts and the exocoelom, the faint rings around the dark spots representing vesicles of the exocoelom beneath, that is dorsal to the angioblasts, if I interpret the drawing correctly.



In spite of the general view that angioblasts differentiate first in the most posterior zone of the area opaca, I am of the opinion that they tend to show first in a more anterior zone, not only in the area pellucida but also in the area opaca. Rückert describes the order as being the reverse in the two areas; that is, first in the posterior part of the area opaca and last in the posterior part of the area pellucida. Throughout the early development angioblasts are more massive in the posterior part of both the area opaca and the area pellucida, but in each area they are always a little farther advanced in the more anterior zone.

At the stage of 2 somites (as shown in fig. 3, plate 1) there is but little change in the mesoderm of the area pellucida, but in the area opaca the great mass of primitive mesoderm has formed large vesicles, the forerunners of the primitive exocoelom. Opposite the middle region of the area opaca, especially, are small masses of dense cells, the first angioblasts. As described by Rückert, these tend to lie opposite the walls of two adjacent vesicles of the exocoelom, so that they alternate with its cavities. I have other specimens of 2 somites which show a greater number of angioblasts in this middle zone, but still the same lack of differentiation in the posterior zone. In one specimen of 2 somites I can identify as angioblasts one or two clumps of cells in the area pellucida; again, specimens of 3 somites may show a few angioblasts in the area pellucida, but usually they are not well marked there until the chick has 4 somites, and I believe are never abundant until the stage of 5 somites is reached.

The stage of 4 somites is especially interesting on account of a differentiation in the zones of the exocoelom. In figure 4, plate 1, from a chick with 4 somites, it will be seen that over the amnio-cardiac vesicles of the area pellucida, and also over the entire anterior half of the area opaca, the layers of cells which make the exocoelom have differentiated into large vesicles. In the posterior half of the area opaca this differentiation is only just beginning, as can be seen in figure 29, plate 6, in which it is clear that along the dorsal border of masses of mesoderm there are two layers of cells, the forerunners of the walls of the exocoelom; while the ventral, solid mass is now made up of angioblasts. Toward the end of the stage of 4 somites this differentiation becomes much more marked. The vesicles of the coelom become larger and the angioblasts more massive. In the area pellucida the zone of the amnio-cardiac vesicles is well marked, as is also the middle zone of the exocoelom, where will be seen the delicate plexus of mesoderm characteristic of this area. In this middle zone are a few angioblasts near the edge of the area opaca. This is the first zone of the area pellucida in which angioblasts appear. The more posterior part of the area shows no differentiation of the mesoderm, except into the more dense axial zone. In the axial line the only indication of the vascular system is a slight thickening of the splanchnic mesoderm which marks the very beginning of the myocardium.

In the history of the vascular system the stage of 5 somites is very important and is shown in two photographs, figures 5 and 6, the former being the less developed. On the left side all of the points are obscured by yolk, but the angioblasts are very clear on the right side. The entire area opaca is occupied by two structures,



the large vesicles of the coelom (which do not show in the photograph) and masses of solid angioblasts. There has been no liquefaction of the angioblasts. The area pellucida has three or four masses of angioblasts in the zone of the amnio-cardiac vesicles, all of them lying opposite the borders of the vesicles of the coelom. Opposite the venous end of the heart in the mid-zone the angioblasts are too delicate to show at this magnification, but they are present, and some of them have even formed tiny vessels. In the posterior zone of the area pellucida the angioblasts are more massive and have already been shown in a drawing to illustrate their contrast to the coelom (fig. 27, plate 6). In the axial line between the myocardium and the edge of the head-fold are a few tiny angioblasts, too small to be seen, which are the forerunners of the endocardium. These are shown in figure 1, in the text, at the stage of 6 somites. Between the myotomes a higher magnification shows the first angioblasts of the aorta.

In contrast to this specimen of 5 somites is one in figure 6, plate 2. In this blastoderm it is clear that the area opaca has now become divided into two zones, an outer and an inner. The two leaders on the left hand indicate the width of the zones. Over the whole outer zone the angioblasts have become transformed into vessels by the liquefaction of most of the angioblasts. This is illustrated in figure 22, plate 5, which is a drawing of the area outlined in figure 6, plate 2. In this drawing it will be seen that there are vessels on the left side forming a blood-island while on the right similar masses of cells lead directly over into a plexus of solid angioblasts which have not yet become vessels. The large blood-islands can be readily compared with the same mass in the photograph. In the specimen it can be noticed that this blood-island is perceptibly darker than the angioblasts just internal to it; this is because the island has enough hemoglobin in its cells to be made out in the counterstain. By a study of the photograph it can be seen that a considerable amount of the original mass of angioblasts has been used up in the formation of the plasma in the outer zone; perhaps less than half of the original mass has remained in the form of blood-islands.

There is undoubtedly a considerable variation in regard to the time at which the angioblasts of the outer rim develop into vessels; in some of my specimens of 6 somites the change has not been made and Rückert (1906) states that vessels with lumina are to be found only in chicks with about 7 somites, and not anywhere in the area vasculosa at the stage of 6 somites (p. 1224, fig. 890). In my specimen of 5 somites, on the other hand, the transformation of the entire mass of angioblasts into vessels in the outer rim of the area opaca has taken place, and I judge that this is about the earliest stage in which one is likely to find the condition. As has been said, in this outer zone it is obvious that the amount of blood-islands left is certainly not more than about half of the original mass of angioblasts, indicating that a considerable amount of the original mass of angioblasts has been used up in the formation of endothelium and plasma. I believe that this is the usual condition in the formation of the early vessels. The vessels will soon fill up almost completely with new masses of blood-cells, but wherever I have observed the actual

liquefaction of the angioblasts in living chick at least half of the cytoplasm has liquefied (fig. 26, plate 6).

In connection with a specimen such as that shown in figure 6, plate 2, it is interesting to consider at what point in the development of the chick the hemoglobin appears. It is, I think, generally admitted that the first masses of cells, which were called blood-islands by the early embryologists, in the stages of 1, 2 and 3 somites have no hemoglobin at all. In the area pellucida in a living specimen one can distinguish hemoglobin by a slight yellow color under the microscope, although the amount is too small to fix and stain. On the other hand, in the cells of the area opaca, which, in these hanging-drop preparations, must always be seen against a background of endodermal cells packed with yolk, one can not be so sure of detecting the first traces of hemoglobin by this color reaction. In specimens of 6 somites, however, in which there has been no liquefaction of the angioblasts to form blood-vessels in the outer rim of the area opaca, it is certain that one can see no traces of the yellow color in the blastoderm in a hanging-drop preparation. In this connection it is interesting to note the work of two Russians on the time of appearance of the hemoglobin. These works I have not seen in the original but know only through a quotation by Maximow (1909, p. 465):

“So fand Smiechowski (1892) dass das Häemoglobin sich optisch und chemisch erst in Hühnerembryonen nachweisen lässt, die schon 12 differenzierte Segmente besitzen. Wulf (1897) der das Häemoglobin speziell mittelst des Spektrokops suchte, fand die ersten Spuren erst beim Hühnerembryo mit 6 Segmenten, während das volle Haemoglobinspectrum erst mit 9 Paar Segmenten erschien.”

The time at which hemoglobin appears, in terms of the number of somites, probably varies within wide limits, but the evidence all tends to indicate that it occurs after the time when the vessels differentiate out of angioblasts, and that hemoglobin is not formed until blood-plasma is produced from the liquefaction of some of the protoplasm of angioblasts. This point seems to me to emphasize again the disadvantage of calling the masses of primitive mesoderm *blood-islands*. All of this evidence is in harmony with the point of view of Madame Danchakoff, now accepted, that in the chick red blood-cells form only within the lumen of a vessel, and suggests at least that the production of some plasma by the liquefaction of certain cells has something to do with the capacity of the cells in the islands to develop hemoglobin. Recently, Madame Danchakoff (1918) has brought forth evidence of the power of endothelium in the chick to produce red cells, even in zones where the endothelium has been so injured that it does not completely inclose a lumen. These observations are especially interesting in connection with the origin of red cells in mammals. It is generally believed that hemoglobin-bearing cells in mammals develop outside of vessels, which would indicate that the theory concerning the importance of endothelium and of plasma in the production of red cells can not be generally applied.

According to Maximow (1909), there are two different types of red blood-cells in mammals: “Sehr merkwürdig ist die Tatsache, dass es beim Säugetier zwei so scharf geschiedene Typen von roten Blutzellen gibt; die primitiven und die

definitiven oder sekundären" (p. 489). He described the first or primitive blood-cells as arising within the vessels in connection with endothelium, like those of birds. These primitive erythroblasts he believes die out completely and are replaced by secondary red cells, which arise from indifferent mesenchyme cells outside of vessels. These mesenchyme cells become lymphocytes, which in turn give rise to erythroblasts (p. 547).

In the article of Mollier (1909), in which he brings out the idea of the extravascular origin of erythroblasts in the developing liver, certain figures (*e. g.*, figs. 7 and 8) can be interpreted easily as a solid mass of angioblasts in which the process of liquefaction is going on with the production of hemoglobin-bearing cells. Indeed, this interpretation also fits in with his conclusions (p. 519):

"In der embryonalen Leber der Säugetiere werden Blutzellen gebildet aus einem indifferenten Material, dem Reticulum, das vom visceralen Blatt des Mesoderms gebildet sich zu Endothelien, Blutzellen und Stützgewebe differenziert. Die Blutzellenbildung erfolgt ausserhalb der Gefässlichtung im Reticulum. Die blutbildenden Gefässanlagen haben alle reticuläre Wand. Die Blutzellen wandern nicht selbständig durch geschlossenes Endothel in die Blutbahn ein, es reisst das Endothel zu diesem Zwecke auch nicht ein, sondern es bleibt die retikuläre Gefässwand solange bestehen, als die Blutbildung anhält."

In studying sections of the developing liver in very young pig embryos, it seems to me that one can identify solid masses of cells between the columns of liver cells as analogous to the solid masses of angioblasts to be seen in blastoderms of the chick. These are the solid, blood-forming capillaries in the sense of O. Van der Stricht. Thus, in view of the great discrepancy in regard to the formation of erythroblasts, namely, that in birds they form within the lumen of vessels and in mammals outside of the lumen, it seems important to test the identification of angioblasts in the case of mammals, especially in connection with Mollier's observation that the walls of vessels in the liver remain reticular as long as blood is being produced there. The question is, therefore, can it be shown for mammals that cells which may be identified as angioblasts produce erythroblasts, and that associated with the process there is a certain destruction, liquefaction, or vacuolation (see Mollier's text-figures 7 and 8) of cytoplasm, such as can be determined for birds?

To return to the specimen shown in figure 6, plate 2, the area pellucida shows three zones: anterior, middle, and posterior. These zones are more striking still in the other photographs on plate 2, especially in figure 7. Over the anterior zone the vesicles of the amnio-cardiac cavities have become very large, with fine, sharp boundaries. Besides these boundaries there are numerous small, isolated clumps of angioblasts. The photograph does not enable one to distinguish these two structures, but they would not be confused in the specimen. Over this area angioblasts are much more scanty than farther posterior, and hence it always happens that they remain very much longer as isolated masses. This is simply due to the fact that the distances between the masses are greater and hence it takes longer for the sprouts to bridge the gaps. When the vacuolation occurs in these isolated clumps there results the formation of isolated vesicles which may be found anywhere in the area pellucida, but more commonly in this region. They were noted in

this area by McWhorter and Whipple in their study on the living chick blastoderm. These authors studied them from the standpoint of the origin of the vessels from tissue-spaces. They are, on the other hand, formed from angioblasts by the liquefaction of the center of the mass and will join the main mass of the vessels by the process of sprouting. At the stage of 5 somites these masses of angioblasts are all solid. Over the middle zone of the area pellucida, which will become the area of the vitelline veins, there is an extensive mass of solid angioblasts, becoming less extensive farther posteriorly. This illustrates that the middle zone opposite the venous area of the heart is always the precocious area in the development of the vascular system in the chick. The myocardium is just indicated in this specimen, and a very few angioblasts, which are the first forerunners of the heart, may be seen between the myocardium and the endoderm, both in the total specimen in figure 6, and in sections from the same stage. Their position is indicated in the diagram of figure 1 in the text, from a chick of 6 somites. No angioblasts along the margin of the somites in the position of the future aorta can be made out in this particular specimen, but they could be in the other specimens of this stage.

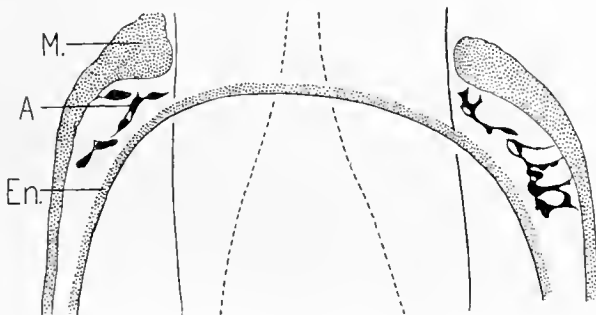
Thus, to sum up the stage of 5 somites, it is important as showing the first blood-vessels from the primitive angioblasts, making an outer rim of vessels over the entire area opaca, the forerunner of the marginal sinus. It marks thus the stage of the first blood-plasma and of the first erythroblasts which can be identified in the form of blood-islands in these primitive vessels. It is the stage in which angioblasts are first found in any great numbers in the area pellucida, as well as in the stage in which the first angioblasts can be seen in the axial line, constituting the forerunners of the endocardium and the aorta.

During the stages of 6, 7, and 8 somites very interesting changes take place. These are illustrated in a blastoderm with 7 somites in figure 7. In the area opaca the blood-vessels of the outer rim have developed great masses of blood-cells. These are no longer in the form of blood-islands attached to the wall, but have become free cells which in many places, and especially at the outer margin, completely fill the lumina of the vessels. In the inner margin of the area opaca, on the other hand, the angioblasts are just beginning to pass into the stage of liquefaction to form vessels.

The area pellucida likewise shows very interesting features. Over the amino-cardiac vesicles, especially of the left side, can be seen fine parallel lines of angioblasts, which are also characteristic of this area. These have been retouched in the photograph. They are on the ventral surface of the amino-cardiac vesicles and are the forerunners of the anterior veins of Popoff. They are especially interesting because one can always find in them examples of the liquefaction of the cytoplasm in single chains of angioblasts, as shown in figure 25, plate 5, which seems to me to be the best proof that the lumen of the vessels may be considered as intracellular. This specimen contains a number of isolated clumps of angioblasts over the dorsal surface of the amino-cardiac vesicles and even extending into the middle zone of the area pellucida. These clumps of angioblasts dorsal to the somatopleure, which are particularly abundant in this specimen, are constant. They are shown in Lillie's

figure 68 *B* (1908) from a chick of 10 somites, and in Van der Stricht's figure 3 (1895, p. 210). In this specimen (fig. 25, plate 5) many of the clumps have become tiny vesicles by the liquefaction of their centers, and several show a few red blood-corpuscles. These vesicles over the somatopleure remain isolated for a long time, simply because they are few and far apart. I have a specimen of the fourth day of incubation, grown on a cover-slip, which has as many as 10 or 12 of them. Some of them, I think, are degenerate, but it soon becomes difficult to follow them in the living specimens on account of the increasing thickness of the blastoderms. Their especial interest lies in noting the early appearance of vessels in the somatopleure in the chick.

In the middle or venous zone of the area pellucida the angioblasts of this specimen have become vessels. They form a delicate plexus and the vessels are consequently small and inconspicuous in the photograph. In the living chick, however, the process of liquefaction can be easily followed. Most of the vessels of this area are empty, that is as far as cells are concerned, but here and there are a few small clumps of red-blood cells, showing that the angioblasts have the potentiality to produce cells bearing hemoglobin. The posterior zone of the area pellucida is especially interesting in this specimen, as it happens to be one in which all of the angioblasts exist in the form of isolated masses of cells. There are numerous delicate sprouts from these masses, but for the most part these have not yet joined similar masses of cells.



TEXT-FIGURE 1.—Diagram showing the position of the angioblasts which are the forerunners of the endocardium in a chick (No. 206) with 6 somites, incubated for 24 hours and 30 minutes and then grown in Locke-Lewis solution in which there was 1.05 per cent NaCl. The diagram shows the actual masses of angioblasts making up the endocardium, and is to be compared with the photograph on plate 1, figure 5.  $\times 135$ . *A*, angioblasts of the endocardium; *En.*, line of the endoderm; *M.*, myocardium.

The stage of 6 somites is the best for studying the differentiation of the heart and aorta from angioblasts. This stage has been extremely well described by Williams (1910-11). Figure 1 in the text shows the edge of the head-fold, and the margin of the myocardium just before it fuses with the symmetrical fold of the opposite side, while between the two are the early chains of angioblasts with tiny vesicles which are destined to make the endocardium of the heart. This figure corresponds to the description of the heart of the Selachian given by O. Van der Stricht (1896). The endocardium continues to receive new angioblasts from the zone of the myocardium, certainly throughout the second day of incubation. These can readily be seen in total mounts of chicks with 17 to 20 somites spanning the wide gap between the myocardium and the endocardium, which is so characteristic of these early stages. The wide gap is not due to shrinkage, because it can be easily

determined with the focusing screw in the living specimen. The endocardium, then, is constantly increased in two ways: first by the division of its cells, and second by the addition of new angioblasts to the outside.

The aorta in the stage of 6 somites looks much like the endocardium shown in text-figure 1, except that the clumps of angioblasts are more isolated. During the stages from 6 to 9 somites the angioblasts along the axial line unite to become a complete vessel. The liquefaction of angioblasts of the dorsal aorta opposite the lower somites can be followed in chicks of 8 to 9 somites, and it is very interesting to note that if a chick of about 9 somites is watched one will usually find a few small blood-islands giving rise to erythroblasts in the lower end of the aorta. At this stage only small islands are formed there, never larger than two or three cells and these from the original angioblasts rather than from a new proliferation of the endothelium.

That the endothelium does proliferate later in the lower aorta to form blood-islands has now been abundantly proved. This was first discovered by Madame Danchakoff (1907) in a study of the development of blood in the chick. She described that in chicks of from 4 to 5 days of incubation there was an intensive growth of the endothelium of the vessels, especially great in the lower aorta, giving clumps of young indifferent elements like the primitive blood-islands. These masses of cells she described as becoming free both within and without the lumina of the vessels, and as giving rise to both red and white blood-cells. In 1909 Maximow described these masses of cells in the aorta of the cat and the rabbit (p. 517), while in 1911 and 1912 they were described by Minot in the human embryo. The question brought up by the latter author as to whether these masses of cells or blood-islands could be proved to be derived from endothelium has, I think, been entirely settled by these observations in the living form. The blood-islands in the aorta have been described more recently by Emmel (1915-1916) in other mammals, especially in pig embryos, and by Jordan (1916-1917) in pig and mongoose embryos. All of these observers describe the islands as giving rise to a primitive mesamœboid cell capable of producing both red and white corpuseles. This point seems to be now the most significant question in regard to these structures. It is generally admitted that in the chick the red cells develop within the lumina of vessels, and I think these observations on the living form make it possible to sharpen this conception by stating that red cells in the chick during the first two days of incubation come either from the original angioblasts or from the subsequent division of endothelium, and that the development of hemoglobin in some cells within the vessels is preceded by the liquefaction of some protoplasm to make plasma. Moreover, it may be said that all of the cells that become free within the lumina of the vessels in the first two days of incubation become red cells. This, of course, does not include certain wandering cells from the endoderm, or germ cells which may get into the vessels. That all of the primitive blood-cells are erythroblasts was pointed out by O. Van der Stricht in 1892 for the chick and in 1895 for the rabbit. Thus the red cells develop intravascularly in the chick because it is angioblasts that give rise to them.

The proliferation of endothelium to make blood-islands, now abundantly proved for the chick and shown to occur in mammals, becomes especially interesting

in connection with the question of the ultimate fate of these cells. All of the cells in these islands in the chick of 2 days of incubation become erythroblasts. All of the observers of this process in mammals agree in considering these masses to be made of primitive hemoblasts or mesamœboid cells, in the sense of Minot, or primitive lymphocytes in the sense of Maximow; that is, capable of producing both red cells and lymphocytes. That they produce erythrocytes is generally conceded, and the evidence that they also produce white corpuscles, that is lymphocytes, is that in many of them none of the signs of hemoglobin can be made out.

The next phases in the development of the vascular system are brought out in two photographs from chicks with 11 somites, figures 8 and 9, plate 2. In both of these figures there is a massive plexus of solid angioblasts in the posterior part of the area pellucida, which is characteristic of this stage. The blastoderm in figure 8, plate 2, had 10 somites when the specimen was taken from the shell, and the heart was just twitching. It shows a great advance over the stage of 7 somites, for the myocardium has fused across the mid-line, the heart has become a vessel, and contrary to the usual form has curved to the left side of the embryo (right side of the photograph). The specimen was fixed during the phase of cell-division for the angioblasts, as is shown in figure 24, plate 5. In this specimen the two sides are not symmetrical in regard to the area opaca. On the right side it will be noticed that there is not a very sharp contrast between the outer and inner rims of the posterior part of the area opaca. This is because the outer rim is full of blood-cells and the inner rim is still made of solid angioblasts. On the left side of the figure there is a part of the inner rim where the angioblasts have become vessels and almost all of the angioblasts have liquefied, the small dark spots representing tiny blood-islands. Figure 9, plate 2, is a photograph of a chick of the same stage, in which all of the angioblasts of the inner rim of the area opaca have liquefied. This specimen also had 10 somites when taken from the shell, and the heart was just twitching; it stopped beating very soon, but the cells continued to live, as they were in process of division when the blastoderm was fixed. The specimen has some abnormalities of the brain and several defects in the mesoderm, but it is very striking for the mass of angioblasts in the posterior part of the area pellucida and for the almost complete liquefaction of the angioblasts of the inner rim of the area opaca. From the study of these living forms, I believe this very complete liquefaction of the angioblasts is the rule rather than the exception—that it to say, it is the rule in the formation of the vessels in these early stages.

To return to figure 8, plate 2, vessels have formed throughout the area pellucida, except in the posterior part, but they are so delicate as not to show in the photograph. They are empty except for a few free blood-cells which have formed in the area since there has been no circulation at this stage. These corpuscles may oscillate back and forth with the beating of the heart, but are not moved through the heart until about the stage of 16 or 17 somites, when the circulation begins. A study of this figure will show that if a transverse section be taken through the posterior part of such an embryo one can see all the processes in the formation of blood-vessels and of



blood-cells in one section. Thus, in the photograph of such a section as that shown in figure 17, it is plain that the vessels of the outer rim, constituting the sinus terminalis, are well formed and contain free blood-cells. Just internal are vessels with blood-islands attached, while in this section the angioblasts just at the border of the area opaca are in process of liquefaction to form vessels. (This zone, outlined in the photograph, is shown in plate 6, fig. 28.) Within the area pellucida are tiny clumps of solid angioblasts that have not yet begun to liquefy.

Thus we have seen that at the stage of 4 somites the area opaca contains solid masses of angioblasts. At the stages of 5 to 7 somites these angioblasts may be forming vessels in the outer rim of this area, while the stage of from 7 to 11 somites will show this outer rim of vessels filling up with blood-cells and the angioblasts of the inner rim of the area opaca liquefying to form vessels. In the posterior part of the area pellucida, on the other hand, the stages of 5 to 11 somites are marked by the active production of new angioblasts, while at the stages of 6 to 9 somites vessels in the anterior zone can be seen to form from angioblasts.

The next specimen in the series (fig. 10, plate 3) is from a chick of 14 somites and is evidently quite characteristic for this stage, as it is so much like Lillie's figure 45 (1908, p. 88) and Rückert's figure 886 (1906, p. 1214). This specimen represents a stage before the circulation has begun, although the heart was beating vigorously. The entire area opaca is covered with well-formed vessels. In the outer half the terminal sinus, as can be plainly seen, is quite filled up with free erythroblasts, more so in my figure than in the other two. The blood in this peripheral zone is now ready to be moved forward into the venous end of the area vasculosa, which is indeed the first movement of the blood after the circulation begins. The inner margin of the area opaca, on the other hand, shows vessels well formed and practically devoid of free erythroblasts, but with numerous young, attached blood-islands. The actual vessels show better in figure 11, plate 3, from another chick of the same stage. They also show in the same manner in the drawings given by Rückert (1906, figure 886,) and Lillie (1908, figure 45), where the interspaces are pale rings and the vessels are a gray network. This is just the way the vessels themselves appear in the specimens, and at first sight almost everyone takes the interspaces to be the lumina of the vessels—not only in a living blastoderm, but also in the fixed specimen. Having once seen the blood circulating in the vessels, however, one will not be confused on this point. Clinging to the walls of the vessels and projecting into the lumina are numerous small islands. In all four figures these extend well into the region opposite the venous end of the heart.

In figure 10, plate 3, throughout the area pellucida the vessels are well formed except in the posterior region, where they are still solid angioblasts; this is also true of Lillie's and Rückert's figures just mentioned. In none of the three figures are these angioblasts conspicuous, a fact which I interpret to mean that all three specimens happened to be fixed during the resting phase. In my own, I know this to be the case. In this specimen liquefaction is just beginning in the margin of the posterior rim between the area pellucida and the area opaca. In figure 11, plate 3, on the other hand, this posterior zone has well-formed vessels except over the



undifferentiated zone of axial mesoderm, where vessels are forming. The transition is shown in two drawings, figures 23, plate 5, and figure 26, plate 6.

I have given two photographs of blastoderms after the circulation has begun. The first is from a chick with 17 somites (figure 12, plate 3), the second from one with 18 somites (figure 13, plate 3). Both show that the mass of blood in the outer rim of the area opaca is drawn forward into the veins by the beat of the heart. In figure 12, plate 3, there is a new generation of tiny blood-islands just beginning in this depleted area. Such new islands are also shown in figure 21, plate 5, from a section through the marginal vein in a specimen of the same stage, *i. e.*, 17 somites. They show very interestingly that a new cycle of blood-islands is beginning in the outer area; this means that a wave of blood-islands will again sweep across the area vasculosa. In both of the specimens there are masses of free blood in the inner rim of the area opaca. These are due not alone to an old generation of blood-islands just breaking up, but also to the fact that opposite the omphalo-mesenteric arteries, which are clearly seen in the older specimen (figure 13, plate 3), there is some abnormal heaping up of red cells in these chicks that are grown on cover-slips. Both specimens show that now the posterior zone of the area pellucida has vessels throughout the outer rim, the new angioblasts being in the axial zone opposite the undifferentiated mesoderm. These new angioblasts belong to the posterior end of the aorta. In the vessels that lie in the arch of the area pellucida posterior to the omphalo-mesenteric arteries are to be seen the blood-islands. Figure 13, plate 3, shows about the maximum number of islands which I have found in this area. This blastoderm has certain especially interesting points: (1) That there are numerous islands in the arteries in direct line with the active circulation, and (2) that in this particular specimen the zone of new islands extends far forward into the venous portion of the area pellucida.

From the figures representing stages with 11, 14, 17, and 18 somites, it is clear, I think, that any section through the lower end of the spinal cord in any of these stages would show all of the processes by which blood-vessels and blood-cells are formed. Thus, if a section were taken through the lower end of the central nervous system in figure 12, plate 3, one would see three generations of blood-islands. In the vessels of the marginal sinus one would find little new islands with two or three cells, like those in figure 21, plate 5; in the inner rim of the area opaca old islands just about to break up, and in the area pellucida islands intermediate between these two extremes. Thus it is clear that after the vessels have formed in the outer part of the posterior zone of the area pellucida, as at the stage of 17 somites, one wave after another of blood-islands sweeps across the area vasculosa, beginning with the outer margin, so that one can find zones of young islands alternating with zones of old ones. In one of my specimens, in which one of these cycles of blood formation was just beginning, so many new unicellular islands were forming in the area pellucida that it seemed almost as if there was an entire duplication of the endothelium. Again, in some of the specimens of 17 somites a cycle of the islands has just been completed and the walls of the vessels are almost bare of islands. If one considers the posterior half of the area vasculosa during the second day of incu-

bation four zones are to be made out: an outer and an inner zone of the area opaca and an outer and axial zone of the area pellucida. In the outer and the inner zones of the area opaca one will always find two different generations of blood-islands; if one zone has young islands the other will have an older generation. In the area pellucida, during these stages, the observer can follow one generation of islands after another in the vessels of the posterior arch, while the posterior axial zone in the stages which we are considering continues to be an area for the differentiation of new angioblasts with but small contributions to the number of red cells.

At the stage when the omphalo-mesenteric arteries are forming, figure 13, plate 3, another factor must be recognized in the living specimen, namely, that along the vessels the mesenchyme cells begin to form in chains along the outer wall which must be distinguished from the endothelium. These cells represent the addition of mesenchyme to the wall of the vessel in the process of development from a capillary into an artery. They are the forerunners of the media and adventia. In the living chicks of 2 days of incubation I have never observed any indication of an endothelial cell leaving the wall of a vessel, a condition described for later stages by Madame Danchakoff (1909) in connection with the formation of blood-cells.

#### CYCLES IN CELL DIVISION.

These cycles in the development of the vascular system are dependent on the fact of cycles in cell division. In these living specimens it has become clear that, in the case of three tissues at least, there are definite cycles of cell division, *i. e.*, in the nervous system, in the endoderm, and in the vascular system. I have not yet studied the ectoderm in this regard, except in the nervous system, and have not found the process so easy to follow in the mesoderm lining the coelom. In the case of the endoderm attention has already been called to the fact that when the entire endoderm passes into the refractive state of the cytoplasm which precedes cell division, the specimen can not be studied for vessels. If such a specimen is fixed at once nothing in the stained preparation will indicate that the cells were about to divide; but if fixation is delayed until an occasional nucleus can be seen in the metaphase, it will be observed that nearly all of the nuclei in the endoderm show division. I have many specimens showing this cycle of division in the endoderm and in the nervous system. The phenomenon is illustrated in connection with the blood-islands in figures 18 and 19, plate 4. In such specimens as the one shown in figure 18, plate 4, if one watches the living island until all of the cells have divided, the entire island will appear to be of the same size as at the beginning, but every cell will be half as large as it was originally. This appearance proves that all of the cells divide in one cycle, notwithstanding the fact that every nucleus does not show a spindle at exactly the same moment. It shows strikingly also that growth (specifically increase in size) occurs in the phases between cell division. Moreover, if one takes the zones of development which have just been described, namely, the outer and inner zone of the area opaca and the outer and axial zone of the posterior part of the area pellucida, all of the cells—either of angioblasts or of blood-islands, whichever happen to be present in any one of these four zones—will be at the same

phase; that is to say, all dividing, resting, or undergoing liquefaction at the same time. I can not make this out to be true of the endothelium after the vessels have formed. In other words, there is not sufficient change in the appearance of the cytoplasm of the finished endothelium in the living specimen to indicate whether the cells are going to divide or not, and in fixed specimens one finds only scattered nuclei with mitotic figures. However, a specimen in which there are any endothelial nuclei with figures will show many of them. Therefore, recognition of this process of cycles in cell division depends on finding the changes in the cytoplasm which precede the nuclear changes in the living form.

That erythroblasts keep on dividing in cycles after they are free from the islands is suggested by identifying several stages of nuclear figures and young cells half the normal size in the circulating blood; that is to say, if one finds one nucleus in the metaphase there will be many in the same phase; or one may find groups in the metaphase and other groups in the prophase. They serve to emphasize the fact that in an embryo after the stage of 5 somites there are two sources of red blood-cells, the endothelial cells of the vessels and free erythroblasts.

The question as to whether or not the same marked cycles of cell division can be made out for the mesoderm, is an interesting one. In many of the early preparations nearly every cell in the myotomes or very extensive masses of the dense axial mesoderm may be found in division; but I have no specimens proving that the entire mesoderm of the embryo or of the membranes divides at one time, as is the case for the endoderm and the angioblasts. In later stages, when angioblasts are differentiating in large numbers, there may be some difficulty in distinguishing a single angioblast in division from a mesodermal cell in division. This is due to the fact that the cytoplasm of the mesodermal cell rounds up around its nucleus during the phase of division and shows also some increase in density, so that it may simulate the angioblasts. There is no difficulty in detecting a fully differentiated, resting angioblast, nor any clump of two or more angioblasts. A specimen such as that shown in figure 9, plate 2, for example, has a very large number of single dividing cells near dense clumps of angioblasts, which I interpret as cells which are just becoming angioblasts, though it may be admitted that in watching such a living specimen in which there is a question of the differentiation of large numbers of new unicellular angioblasts, the final proof of the nature of the cells would be their behavior after they had finished their first phase of cell-division. Had the cytoplasm remained rounded up, and the new cells remained together, they would soon join a neighboring band of angioblasts; on the other hand, had the two cells separated and put out the delicate exoplasm characteristic of mesoderm, the cells would still be undifferentiated.

A question of great importance is whether or not any red blood-cells can be seen to differentiate outside the lumen of a vessel. All of my evidence tends to show that in these stages the red cells develop only within the vessels. This is in entire agreement with the view of Madame Danchakoff (1909), who has shown that in the chick the development of the red cells is intravascular, while that of the granular, white corpuseles is extravascular. In the living blastoderm the only

specimens in which the matter could be called into question is such a one as that just described, in which there are many cells that I term unicellular angioblasts. These can always be found at the stage of 11 somites in the posterior part of the area pellucida. When such a blastoderm is fixed one might bring up the question as to whether any of the cells outside the vessels might not contain hemoglobin, but I think that the answer is negative, on the ground that at this stage no hemoglobin-bearing cells are forming within the vessels of this area. The question could be definitely settled by more careful records than I have yet made of the evidences of hemoglobin in the living cells.

In later stages, on the other hand, after a considerable mass of blood has been formed, the presence of true erythroblasts in the interspaces is a very common occurrence in all of the specimens, just as it is a familiar phenomenon in all sections of embryos. I interpret these cells as having escaped from the lumina of the vessels, just as they have been interpreted in the study of sections, and for the same reasons. In the first place, there must be some rupturing of the delicate walls of endothelium in the mounting; often the specimens must be shaken a little in the solution to free them from the vitelline membrane, and it is hardly possible that any artificial medium would not make some change in these thin membranes. Then, just as Madame Danchakoff (1909, p. 125) finds in sections, I find in the living specimens that these cells tend to degenerate. Moreover, I have never observed them to move toward the vessels, indicating that, like the erythroblasts within the vessels, they have but little motility. The question as to whether any red cells of the chick ever differentiate outside the lumen of a vessel is one of very considerable importance on account of the theory that they do so differentiate in mammals. So far the evidence in the study of these living forms is that in the chick erythroblasts differentiate only within the lumen of a vessel. I have made but few specimens of 3 or 4 days of incubation. The method can be applied up to the fourth day, and in one such specimen there is an isolated vesicle near one of the branches of the artery which is packed with erythroblasts, showing that the formation of new angioblasts giving rise to vesicles is going on, and again emphasizing the intravascular formation of the red cells.

Another point of significance, which these observations on the living specimens seems to me to settle definitely, is that all of the cells of the blood-islands of the chick during the first two days of incubation become red cells; that is, they all develop hemoglobin. This is very striking in the living chick in which every single cell of an island can be seen to be yellow, and all of the cells of a given island are uniformly yellow. In fact, all of the cells of these islands appear alike except for an occasional dead cell among them. These, as has been said, react to neutral red, and in fixed preparations show picnotic and fragmented nuclei. Thus the only cells which by any chance could be confused with white blood-cells can be proved to be dead cells. The granular corpuscles can be identified in the blood-vessels in the living chick at the stage of the fourth day of incubation and their origin must therefore be taken up in later stages.

## ORIGIN OF THE HEART AND AORTA.

It may be interesting to sum up what can be seen of the origin and development of the heart and the aorta in these living blastoderms. As has been shown, it is possible to find the first angioblasts that differentiate from mesoderm in the wall of the myocardium before the two myocardia have fused across the mid-line. These cells appear first at the stage of 5 somites. At the same time isolated clumps of cells appear along the ventro-lateral margin of the somites, the forerunners of the aorta. These clumps of isolated angioblasts liquefy to form vesicles, which gradually unite to form the endocardium and the aorta. By the time the chick has 9 somites a complete aorta can be seen along the ventro-lateral margin of the somites, but this vessel is constantly increased by the addition of new angioblasts. The stage of 9 somites is especially favorable for watching this process. Indeed, I have found it rare that a chick of that stage does not show a few clumps of angioblasts joining the aorta along its mesial border opposite the last two somites. Moreover, practically all chicks with from 14 to 17 somites will show new angioblasts joining the aorta opposite the undifferentiated mesoderm at the lower end of the embryo. Therefore, any illustrations of an injection of the lower end of the aorta (such as are shown in Evans's [1909] fig. 1) should have added to them a few masses of solid angioblasts about to join the wall of the plexus in order to completely represent the aorta in that area. It is interesting to note that in this area, when a mass of angioblasts joins the wall of a vessel, sprouts from the older vessel and from the young masses of cells meet half way between the two structures, and then the new cells seem to be gradually drawn into the vessel wall. On the other hand, in the growth of a plexus the new cells may remain at some distance from the older vessel, while the protoplasmic bridge develops into a vessel connecting the two lumina. This is a different process from the drawing of new cells into the wall of a vessel, such as can be seen so readily in the case of the endocardium and the wall of the aorta.

The study of the heart in these specimens is interesting. The early stages have already been mentioned. There are at first isolated clumps of angioblasts which form tiny vesicles, as shown in text-figure 1. These vesicles then unite to make a plexus. The development of the later stages must be watched through the myocardium and hence it can not be seen as clearly as the vessels themselves. After the endocardium has formed a complete vesicle the heart curves usually to the right, by an occasional anomaly to the left, as seen in figure 8. The very first beats of the heart can be made out in these hanging-drop specimens. They occur at the stage of 10 somites and always in the same position. The first twitching is along the right margin (left side of the photograph), beginning just at the lower border above the junction and between the vitelline vein and the ventricle. It is interesting to note that there is no movement whatever in the vein, the entire twitching being confined to the ventricle proper. That is to say, the myocardium over the ventricle is formed long before there is any muscle at all in the wall of the veins. The beat is at first slow but rhythmical, and gradually involves the entire wall of the ventricle, spreading from the posterior to the anterior end. The heart beats throughout the stages of from 10 to about 16 somites without actually pumping any blood into the aorta. At the stage of 16 to 17 somites circulation begins.

## CONCLUSIONS.

These studies have shown that throughout the first two days of incubation there is a continual differentiation of mesenchymal cells into angioblasts. In the chick these cells arise first in the embryonic membranes, but by the stage of 5 somites they begin to differentiate actively in the embryo itself and both the endocardium and aorta can be seen to differentiate *in situ*. Moreover, angioblasts continue to differentiate in the wall of the yolk-sac during the third and fourth days of incubation.

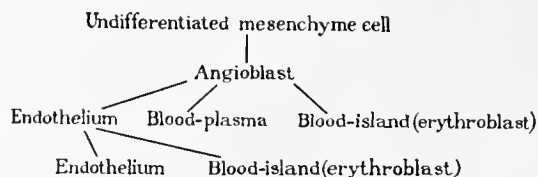
The characteristics of these new angioblasts have been made sufficiently sharp so that they can be identified in sections. In this way we can be sure that not only the aorta but the primitive vessels of the embryo, such as the vessels along the nervous system and the cardinal veins, differentiate *in situ*. These studies must leave open the question as to the time, if ever, when angioblasts cease entirely to differentiate and all of the growth comes to be from the walls of previously formed vessels, as was observed by Clark (1909) for the tadpole's tail. The place at which to attack this problem again seems to me to be in further studies on the regeneration of vessels in healing wounds.

In these observations on the living blastoderm of the chick it has been shown that it is possible to make a sharper distinction between the cells which differentiate to form the coelom and those which form the vascular system. It therefore seems better to use the term *primitive mesoderm* for the masses of cells which will give rise to both structures. Indeed, the relationships can be made quite clear by limiting the term *blood-island* to those masses of cells that actually develop hemoglobin and become erythroblasts, while the indifferent masses, which will give rise to mesoderm on the one hand and angioblasts on the other, are given a less specific name.

The exocoelom forms by a splitting apart of the two layers of cells which come from the primitive mesoderm. Blood-vessels, on the other hand, arise by the differentiation of a new type of cell from this same primitive mesoderm. This has a different cytoplasm from the original mesodermal cell, is more granular, more basophilic, and has new qualities, namely, the tendency to form solid masses which appear like a syncytium, the centers of which liquefy to form blood-plasma, and a marked tendency to put out delicate sprouts by which it joins similar masses. These cells, or angioblasts, give rise to endothelium, blood-islands, and blood-plasma.

All of the cells of the blood-islands of the first two days of incubation become erythroblasts. The lumina of the vessels is not formed of tissue-spaces, but rather by a process of cytolysis in the center of masses of cells or even within the cytoplasm of a single cell. An endothelial cell differentiates in its turn from the original angioblast. It can give rise to other endothelial cells or to erythroblasts.

The observations herein recorded do not bear on the question of the origin of white blood-cells, because there are no cells in the chick of the second day of incubation that can be identified as the ancestors of the white cell. They do show, however, that the ancestry of the red cells can be outlined as shown by the diagram above.



In this scheme I have used the term *blood-island* in the sense of hemoglobin-bearing cells attached to the inner wall of a vessel, while *erythroblast* is used in the usual sense of free hemoglobin-bearing cells that are continuing to divide. For the chick the question concerning the origin of the white cells must be concerned with two possibilities; first, whether all of the white cells develop subsequently from the mesenchyme outside the vessels that does not go through the stage of differentiating into angioblasts; second, whether in later stages the endothelium produces true lymphocytes that do not develop hemoglobin.

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## LIST OF ILLUSTRATIONS.

### PLATE 1.

- FIG. 2. Blastoderm of a chick (No. 141) with 1 somite, incubated for 28 hours and then grown for 22 minutes in Locke-Lewis solution in which there was 1.06 per cent of NaCl. It had no somites when taken from the shell. The specimen is viewed from the endodermal surface, as is the case for all of the photographs on plates 1, 2, and 3. The lines of the endodermal blisters in the posterior part of the area pellucida on the right side have been retouched on the photograph. The line across the figure is approximately the level of the section shown on plate 4, figure 14, from a chick of about the same stage and passes through the endodermal blisters.  $\times 24$ . S. 1, first somite.
- FIG. 3. Blastoderm of a chick (No. 212) with 2 somites, incubated for 25 hours and then grown for 3 hours and 45 minutes in Locke-Lewis solution containing 1.05 per cent NaCl and only 0.014 per cent of  $\text{CaCl}_2$ . It had no somites when taken from the shell; 2 are shown faintly in the photograph.  $\times 30$ . A., angioblasts; S. 1, first somite.
- FIG. 4. Blastoderm of a chick (No. 201) with 4 somites, incubated for 26 hours and 40 minutes and then grown for 35 minutes in Locke-Lewis solution having 1.06 per cent NaCl and only 0.025 per cent of KCl. It had 3 somites when taken from the shell. The more anterior line across the figure is approximately the level of the section shown on plate 4, figure 15, and passes through the large vesicles of the mesoderm characteristic of the amnio-cardiac vesicles. The more posterior line shows the level of the section on plate 6, figure 29.  $\times 24$ .
- FIG. 5. Blastoderm of a chick (No. 198) with 5 somites, incubated for 26 hours and then grown for an hour in Locke-Lewis solution containing 1.06 per cent of NaCl and only 0.025 per cent of KCl. It had 4 somites when taken from the shell. The line across the figure shows the level of the section shown on plate 4, figure 16, from another specimen of about the same stage. It passes through the delicate network of the coelom characteristic of the middle and posterior part of the area pellucida. The square on the right side shows the zone which has been drawn for plate 6, figure 27, to show the appearance of angioblasts against the delicate network of mesoderm characteristic of the coelom. On the left side of the photograph there is a large amount of free yolk. Stained with hematoxylin alone.  $\times 18$ .

### PLATE 2.

- FIG. 6. Blastoderm of a chick (No. 213) with 5 somites, incubated for 25 hours and 10 minutes and then grown for 3 hours and 45 minutes in Locke-Lewis solution containing 1.05 per cent NaCl and only 0.014 per cent of  $\text{CaCl}_2$ . It had 3 somites when taken from the shell. It shows especially well four zones in connection with the vascular system: (1) an outer zone of the area opaca, indicated by the leader No. 1, in which all of the angioblasts have become vessels; (2) an inner zone of the area opaca, indicated by the leader No. 2, in which the angioblasts are still solid; (3) an outer zone of the area pellucida with solid angioblasts; (4) an axial zone of dense mesoderm. The rectangle on the right side shows the area which was drawn for plate 5, figure 22, inclosing the transition between the outer and inner zones of the area opaca.  $\times 18$ .
- FIG. 7. Blastoderm of a chick (No. 238) with 7 somites, incubated for 29 hours and 30 minutes, and then grown for 35 minutes in Locke-Lewis solution in which there was 1.06 per cent of NaCl. It had 6 somites when taken from the shell. It shows a number of clumps of quite isolated angioblasts in the posterior part of the area pellucida, and over the amnio-cardiac vesicles there are the long, slender chains of angioblasts characteristic of that region. They have been retouched in the photograph.  $\times 15$ .
- FIG. 8. Blastoderm of a chick (No. 177) with 11 somites incubated for 52 hours and 10 minutes and then grown for 3 hours and 35 minutes in Locke-Lewis solution containing 1.06 per cent of NaCl and 0.052 per cent of KCl. It had 10 somites when taken from the shell and the heart was just twitching along the outer border. It shows masses of solid angioblasts in the posterior part of the area pellucida which are in the phase of division. Contrary to the usual position, the heart projects to the left side of the embryo, right side of the photograph. The rectangle indicates the position of plate 5, figure 24.  $\times 15$ .
- FIG. 9. Blastoderm of a chick (No. 151) with 11 somites, incubated for 42 hours and 40 minutes and then grown for 3 hours and 40 minutes in Locke-Lewis solution in which there was 1.06 per cent of NaCl. It had 10 somites when taken from the shell and the heart was just twitching. The specimen shows an abnormal form of the brain and there are two large defects in the mesoderm, one on the right side and the other at the posterior end. It is especially fine for the plexus of solid angioblasts in the posterior part of the area pellucida; in fact shows the maximum amount of angioblasts in any of my specimens. It also shows the outer and inner zones of the area opaca especially well, for the vessels of the outer zone are packed with blood-cells and the angioblasts of the inner zone have just liquefied. The line across the figure shows the level of plate 4, figure 17.  $\times 17$ .

### PLATE 3.

- FIG. 10. Blastoderm of a chick (No. 150) with 14 somites, incubated for 42 hours and then grown for 2 hours in Locke-Lewis solution in which there was 1.06 per cent of NaCl. It had 11 or possibly 12 somites when taken from the shell and now the fourteenth is just indicated in the undifferentiated mesoderm. From this stage on the number of somites anterior to the cardiac fold can not be made out for certain in the living chick and records should state the number posterior to the cardiac fold. The heart was beating but there was no circulation. The photograph shows four zones in connection with the vascular system, in a transverse line just posterior to the dilated end of the spinal cord; an outer zone in the area opaca in which the vessels are packed with free red blood-corpuscles; an inner zone in the area opaca in which there are blood-vessels containing blood islands; an outer zone of the area pellucida with solid angioblasts; and an axial zone in which there are more delicate angioblasts which do not show at this magnification.  $13\times$ .

## PLATE 3—continued.

- FIG. 11. Blastoderm of a chick (No. 174) with 14 somites, incubated for 52 hours and 45 minutes and then grown for 2 hours and 15 minutes in Locke-Lewis solution having 1.06 per cent of NaCl and only 0.014 per cent of  $\text{CaCl}_2$ . It had 12 somites when taken from the shell. It shows the plexus of vessels in the entire area pellucida and demonstrates that the blood-islands in the posterior part of the area pellucida are within the vessels. The interspaces are pale rings and the vessels are a gray plexus, while the dark spots in the plexus are the blood-islands. The heart was beating but there was no circulation, and hence the veins are empty except for a few clumps of red cells, which have developed in them. In the vitelline veins close to the heart the streaks are due to the fact that the interspaces are so close that the endothelium of two adjacent vessels touch and therefore appear as lines. The rectangle covers the area from which two drawings were made, namely figures 23 and 26.  $\times 11$ .
- FIG. 12. Blastoderm of a chick (No. 145) with 17 somites, incubated for 43 hours and 15 minutes and then grown for 2 hours in Locke-Lewis solution having 1.06 per cent of NaCl. It has blood-vessels throughout the area pellucida. This is the specimen in which the islands were drawn in the living form shown in figure 18 from the zone indicated by the line No. 1. It had 17 somites when taken from the shell and the number did not increase. The heart was beating and the circulation had begun, but was not reestablished on the coverslip. It is also the specimen in which the blood of the outer zone of the area opaca has been drawn forward into the veins, and in which a generation of new blood-islands is beginning in this area.  $\times 8.6$ .
- FIG. 13. Blastoderm of a chick (No. 183) with 18 somites, incubated for 52 hours and 45 minutes, and then grown 4 hours in Locke-Lewis solution containing 1.06 per cent of NaCl and only 0.014 per cent of  $\text{CaCl}_2$ . It had 18 somites when taken from the shell and the number did not increase. The heart was beating vigorously and the circulation was reestablished on the coverslip. It shows three areas in which blood-cells are somewhat abnormally massed; first, in the anterior veins; second, in the vitelline veins close to the heart; third, opposite the mesenteric arteries. It has blood-vessels throughout the area pellucida and shows a large number of blood-islands in the posterior part of the area pellucida.  $\times 7.2$ .

## PLATE 4.

- FIG. 14. Photograph of a section through a blastoderm (No. 144) with no somites to show endodermal blisters. The section passes through the lower part of the primitive streak at approximately the level of the transverse line on plate 1, figure 2. The chick was incubated for 26 hours and 45 minutes, was then grown for 2 hours and 10 minutes in Locke-Lewis solution having 1.06 per cent of NaCl, and fixed while a few endodermal blisters were present. There is a small blister on the left side which contains a wandering endodermal cell and a larger empty one on the right side. The mesoderm is in solid undifferentiated masses close to or touching the ectoderm.  $\times 60$ .
- FIG. 15. Photograph of a section through the amnio-cardiac vesicles of a chick (No. 115) with 3 somites which had been incubated for 23 hours and 25 minutes and then grown for 2 hours in Locke-Lewis solution. It had 3 somites when taken from the shell and the number did not increase. The section is taken at approximately the level of the line across plate 1, figure 4. The section shows an open neural tube. The large, closely packed vesicles characteristic of the development of the amnio-cardiac vesicles are clearly shown, with small clumps of angioblasts opposite the walls of these vesicles.  $\times 60$ . A, angioblasts; C, coelom.
- FIG. 16. Photograph of a section through the first somite from a blastoderm (No. 155) with 5 somites, incubated for 27 hours and 20 minutes and then grown for 2 hours in Locke-Lewis solution having 1.06 per cent of NaCl. It was taken at approximately the level of the transverse line on plate 1, figure 5, which is a specimen of the same stage. The section shows the type of the coelom, which is characteristic of the middle and posterior zones of the area pellucida. In the area pellucida the wide gaps between the vesicles of the coelom, where there are no cells except the ectoderm and endoderm, are very plain.  $\times 60$ .
- FIG. 17. Photograph of a section of a blastoderm (No. 95) with 11 somites, incubated for 42 hours and 30 minutes and then grown for 2 hours in Locke-Lewis solution. It had 9 somites when taken from the shell. The photograph is given especially to show the position of the drawing on plate 6, figure 28, which is taken within the square. It shows all the processes of formation of the blood-vessels and blood-islands in a single section. At the outer edge of the area opaca are blood-vessels, the sinus marginalis containing free blood-corpuscles. Within the square at the inner border of the area opaca are both blood-islands in a vessel and angioblasts liquefying to form the lumen of a vessel, while in the area pellucida are new clumps of angioblasts.  $\times 48$ . A, angioblasts; l, lumen of the sinus marginalis.
- FIG. 18. Blood-islands in the vessels of the area pellucida of a chick (No. 145) of 17 somites, drawn from the living specimen. It is designed to show as nearly as possible the appearance of the living tissues. The region from which the drawing was taken is shown by the leader No. 1, plate 3, figure 12, from a photograph of the same specimen, but which does not show the exact form of the drawing since the specimen was fixed 2 hours and 15 minutes after the outlines for the drawing were made. The lumina of the vessels is made to appear like ground glass, which represents their actual appearance. The small drawing at the bottom of the figure shows the phase of division of the nuclei, which took place while the drawing was being made. At the top of the figure are two unicellular blood-islands and some free red blood-corpuscles in the lumen of the vessel. On the right side is a large blood-island attached to the endothelium of the vessel.  $\times 450$ . B, *i.*, young blood-island shown in the resting phase, the small drawing below being the same island during division; e., endothelium; *i.*, interspace between the vessels as it appears in the living specimen; it represents the mesoderm beneath the vessels, not analyzed with reference to its cells, and is bordered with a rim of endothelium; l., lumen of a vessel.

## PLATE 4—continued.

- FIG. 19. Blood-islands from the area pellucida of a chick (No. 135) with 19 somites, drawn to show the appearance of the living specimen. The chick was incubated for 42 hours and 20 minutes in Locke-Lewis solution having 1.07 per cent of NaCl. The heart was beating and the circulation was established. The islands were taken from the posterior part of the area pellucida at about the same region as the drawing of figure 18 of the same plate. The specimen shows a late stage in the development of the islands; the cell outlines are an indication that the islands were undergoing division, though the chromosomes were not seen in the living specimen. In the fixed specimen more than half of the nuclei are in the prophase, which can not be made out in these thick specimens while living.  $\times 450$ . *B. i.*, blood islands; *e.*, endothelium; *i.*, interspace; *l.*, lumen of the plexus of vessels.
- FIG. 20. Plexus of angioblasts from a chick (No. 161) with 13 somites, drawn to show the appearance of the living specimen. The chick had been incubated for 50 hours and then grown for 3 hours and 45 minutes in Locke-Lewis solution in which there was 1.06 per cent of NaCl. It had 12 somites when the egg was taken from the shell and the drawing was made at that stage. The heart was beating, but there was no circulation. The drawing is to be compared with the blastoderms shown on plate 2, figures 8 and 9, and on plate 3, figure 10; and is from the zone just lateral to the rectangle shown on plate 2, figure 8. The drawing shows the character of the bands of angioblasts before there is any liquefaction whatever as seen in the living chick.  $\times 475$ . *A*, angioblasts; *i.*, interspace.

## PLATE 5.

- FIG. 21. Section through the marginal sinus of a chick (No. 96) with 18 somites, which had been incubated for 43 hours and then grown for 2 hours in Locke-Lewis solution. It had 17 somites when taken from the shell, and when fixed the eighteenth was just appearing. It is to show a new generation of blood-islands beginning in the marginal sinus, like those of the total preparation shown on plate 3, figure 12. The heart was beating and the circulation well established. The ectoderm is not included in the drawing.  $\times 700$ . *B. i.*, unicellular blood-island in the edge of the marginal sinus; to the left is a larger blood-island, also attached to the endothelium, and the sinus has many free erythroblasts; *e.*, exocoelom; *en.*, endoderm; *en. c.*, endodermal cell full of yolk and ready to become a wandering cell; *mes.*, mesoderm along the border of the exocoelom.
- FIG. 22. Drawing of the transition zone between the outer and inner zones of the area opaca in a chick (No. 213) with 5 somites. The total blastoderm from which the drawing was taken is shown on plate 2, figure 6. The place of the drawing is shown by the rectangle in the photograph, but the position is reversed. On the left side, outer zone of the embryo, the vessels have formed and show blood-islands attached to their walls, while on the right side the hollow vessels lead over into the plexus of solid angioblasts.  $\times 330$ . *A*, angioblasts, the suggestion of cell outlines indicating cell division; *b. i.*, blood-island within the lumen of the vessel; *i.*, interspace; *l.*, lumen of a vessel.
- FIG. 23. Drawing of a mass of angioblasts in which the liquefaction of the cytoplasm is very extensive, from a chick (No. 174) with 14 somites. It is from the blastoderm shown on plate 3, figure 11. Both this figure and the one on plate 6 (fig. 26) were taken within the rectangle on the photograph. They are both vessels which make up the lower end of the developing aorta. This figure shows a mass of angioblasts which is going to liquefy entirely, while the other shows a partial transformation of angioblasts into erythroblasts. The vacuoles vary greatly in size, and many of them are immediately against the nuclei.  $\times 920$ .
- FIG. 24. Drawing of solid angioblasts from the posterior part of the area pellucida from a chick (No. 177) with 11 somites. It is from the blastoderm shown on plate 2, figure 8, the position of the drawing being shown by the rectangle on the photograph. The drawing is reversed from the photograph. The angioblasts are entirely solid and show no sign of liquefaction. The mass was undergoing division and different types of nuclear figures are plain. At the lower left-hand border a single nucleus has elongated to make a typical endothelial nucleus, while on the right-hand border a single cell has become an angioblast and is about to join the main mass.  $\times 920$ .
- FIG. 25. Drawing of angioblasts taken during the process of liquefaction from a chick (No. 172) with 13 somites. The specimen had been incubated for 48 hours and 30 minutes in Locke-Lewis solution having 1.06 per cent of NaCl and only 0.014 per cent of  $\text{CaCl}_2$ . The drawing is taken from the arch of the area pellucida posterior to the end of the spinal cord in a specimen nearly like that on plate 3, figure 10. The chick had 11 somites when taken from the shell and the heart was just beating. The entire center of the mass is solid, but there are vacuoles forming along the edges, leaving an endothelial border. In the upper right-hand process the lumen of the vessel is forming within a single angioblast between two nuclei.  $\times 920$ .

## PLATE 6.

- FIG. 26. Drawing of a vessel in which a part of the original angioblasts has developed hemoglobin and is thus forming blood-islands instead of liquefying; from a chick (No. 174) with 14 somites. It is from the blastoderm shown on plate 3, figure 11. Both this figure and that shown on plate 5 (fig. 23) were taken within the rectangle on the photograph. They are both vessels that form a part of the lower end of the developing aorta. The clump of two cells to the right are new angioblasts; in the specimen they are along the vessel wall just outside of the area drawn, but are about the same distance from the main vessel; they were shifted in the drawing, so as to come within the field.  $\times 920$ .
- FIG. 27. Drawing of a plexus of angioblasts as seen against the developing coelom in the area pellucida of a chick (No. 198) with 5 somites. It is from the blastoderm shown on plate 1, figure 5, and covers the area of the square on that photograph. The angioblasts are shown as dense bands against the more delicate background of the coelom. In several places there are transition zones between the two structures. There are numerous gaps in the mesodermal layer.  $\times 400$ . *A*, angioblasts; *mes.*, mesoderm.

## PLATE 6—continued.

- FIG. 28. Section through the inner edge of the area opaca of a chick (No. 95) with 11 somites, which was incubated for 42 hours and 30 minutes and then grown for 2 hours in Locke-Lewis solution. The chick had 9 somites when taken from the shell, and the eleventh was just appearing when it was fixed. The heart was not beating when the egg was opened, but twitched slightly on the coverslip. The section is approximately at the level of the line across plate 2, figure 9, which is a specimen of about the same stage. A photograph of the section from which the drawing was made is given on plate 4, figure 17, with a square to indicate the zone. It shows the process of liquefaction of angioblasts to make vessels, as it can be seen in sections, as well as a blood-island attached to the endothelium of the same vessel. It shows the same processes illustrated in total preparations on plate 5 (fig. 23) and plate 6 (fig. 26).  $\times 700$ . *A*, angioblasts in the process of liquefying, showing pyknotic nuclei; *b. i.*, blood-island; *c*, exocoelom; *ec.*, ectoderm; *en.*, endoderm; *L*, lumen of vessel; *mes.*, mesoderm.
- FIG. 29. Section through the posterior part of the area opaca of a chick (No. 148) with 4 somites, which had been incubated for 30 hours and 15 minutes, and then grown for an hour in Locke-Lewis solution having 1.06 per cent of NaCl. It is taken at approximately the level of the lower line on plate 1, figure 4, from a specimen of about the same stage. The drawing shows the differentiation of the primitive mesoderm into the two layers of cells which form the coelom and the more ventral solid clumps of angioblasts.  $\times 620$ . *A*, angioblasts; *c*, coelom; *ec.*, ectoderm; *en. c.*, endodermal cell which has wandered to a position dorsal to the mesoderm; *mes.*, mesoderm.

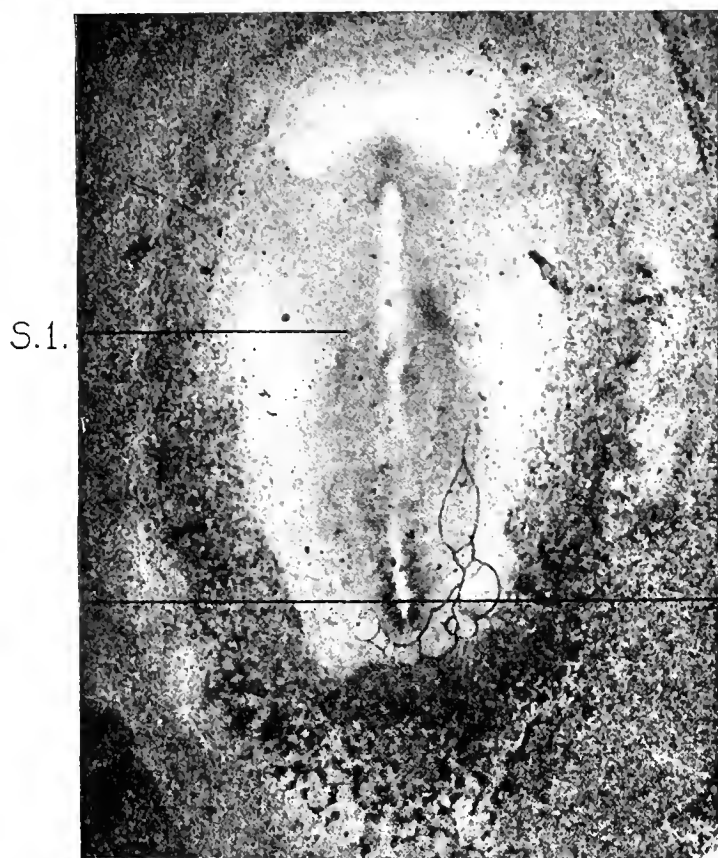


Fig. 2.

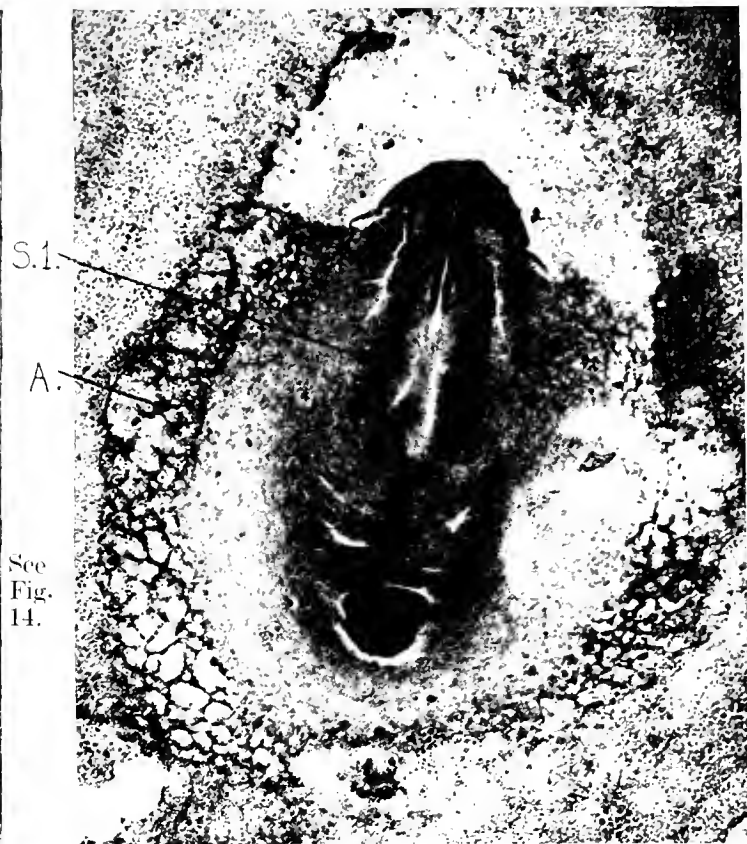


Fig. 3.



Fig. 4.

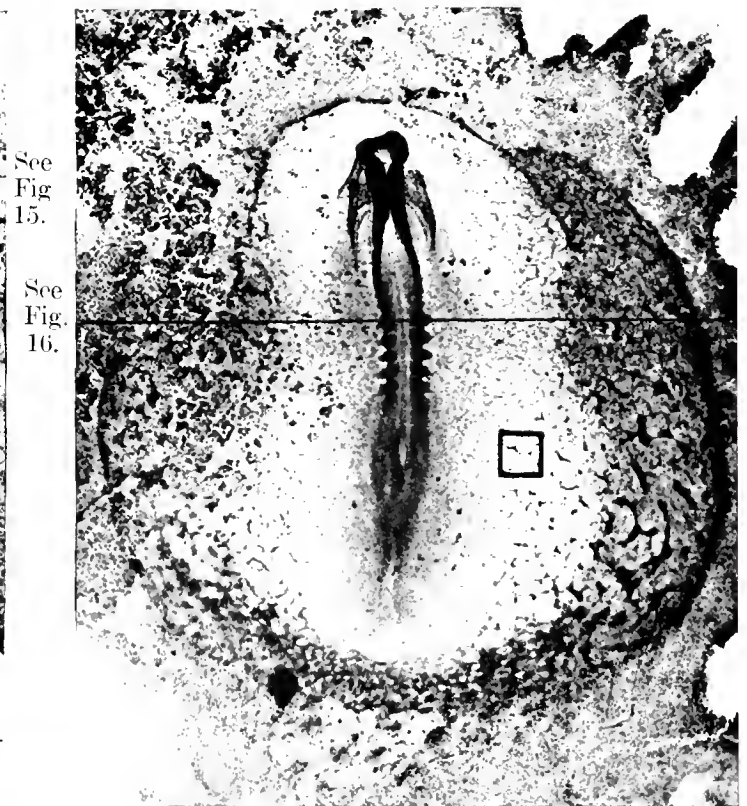


Fig. 5.







Fig. 6



Fig. 7



Fig. 8

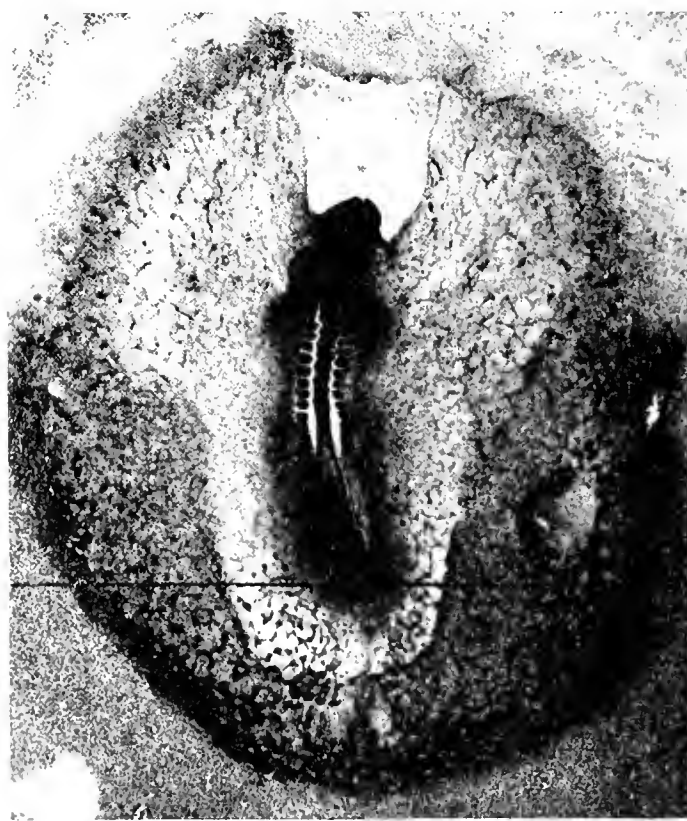


Fig. 9





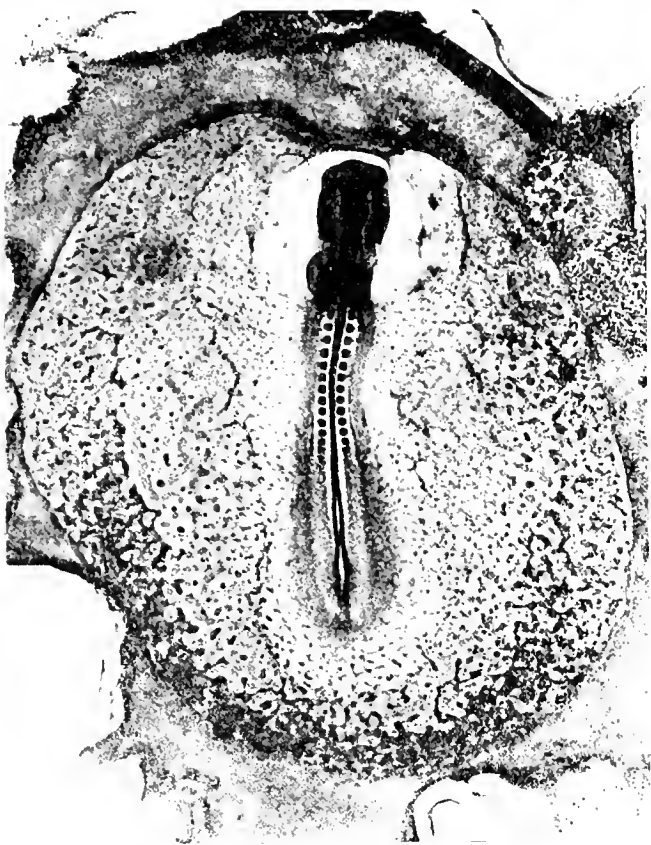


Fig. 10



Fig. 11

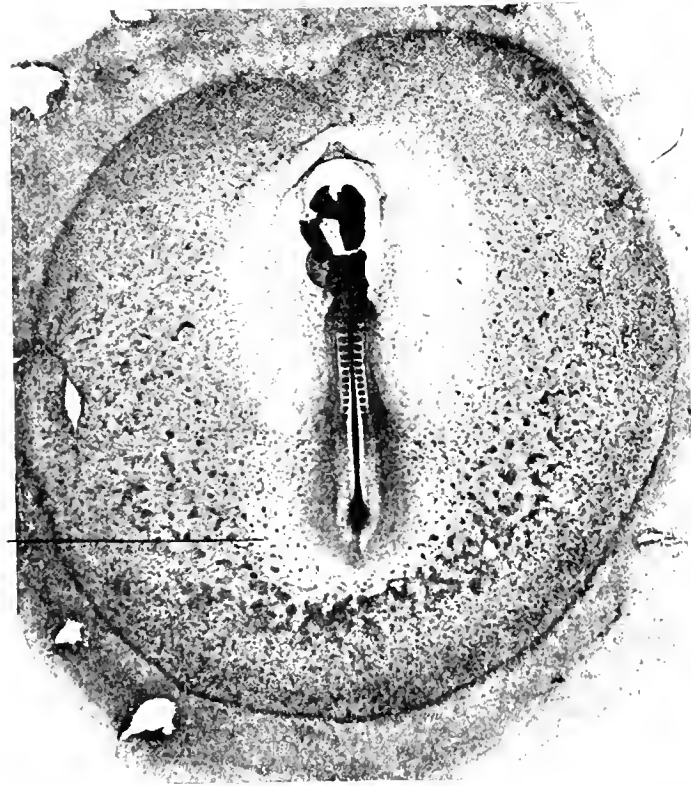


Fig. 12

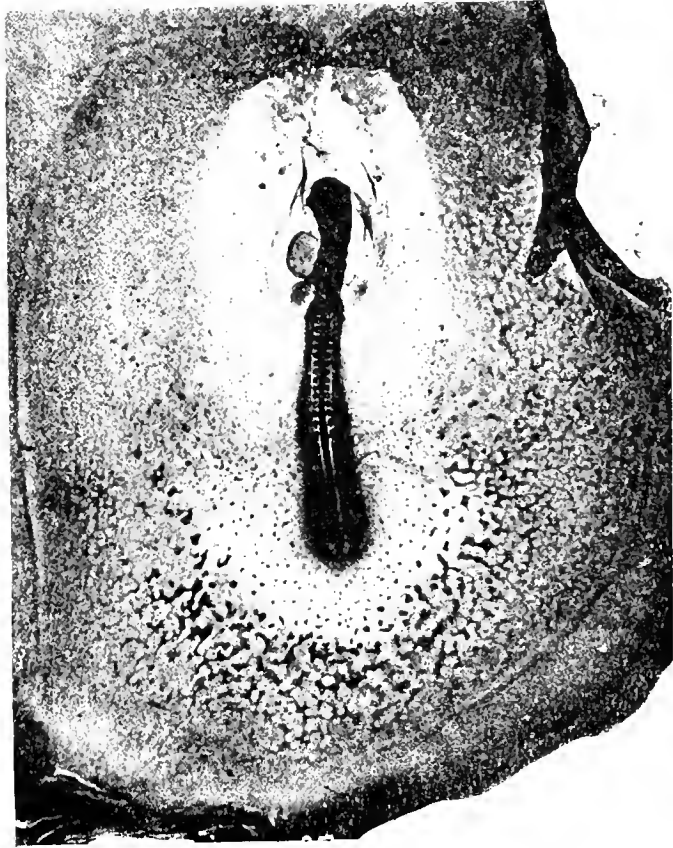
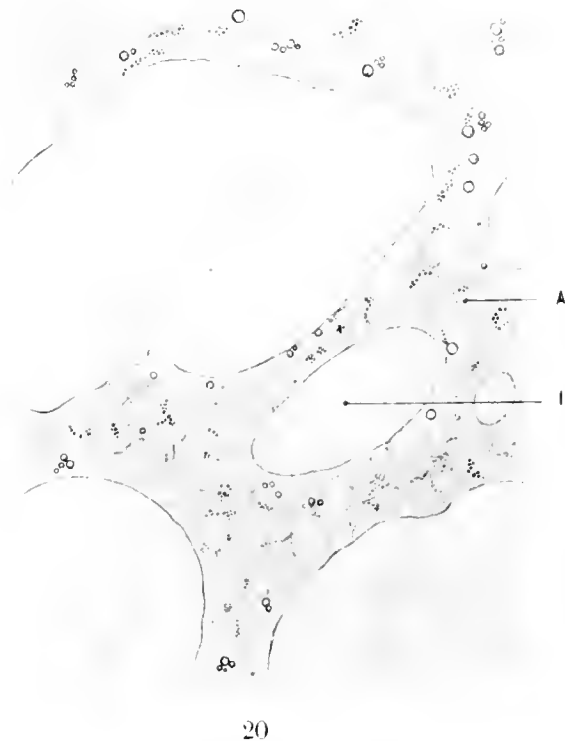
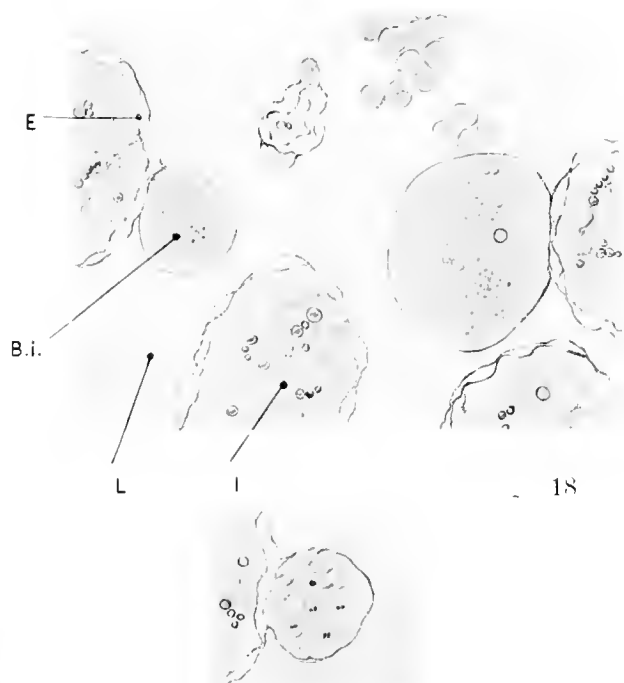
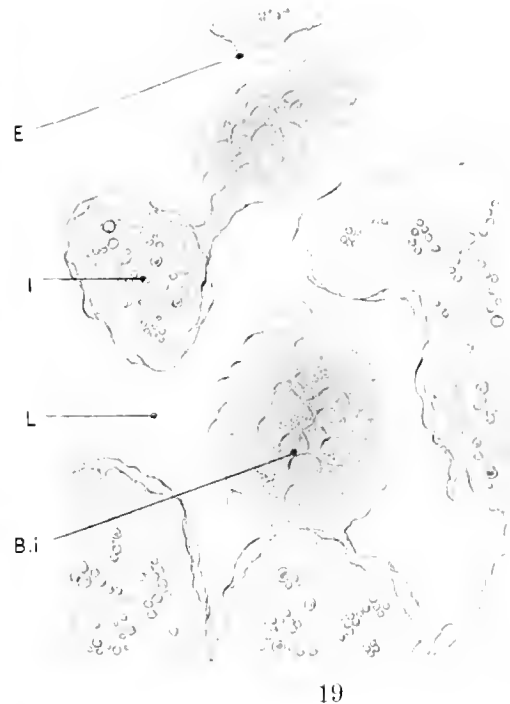
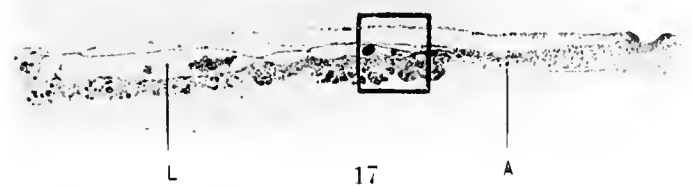
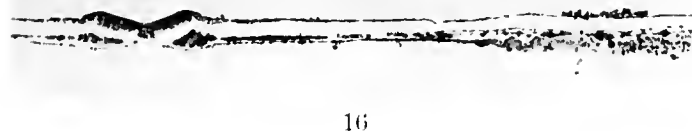
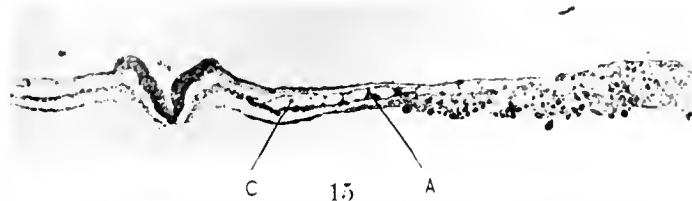
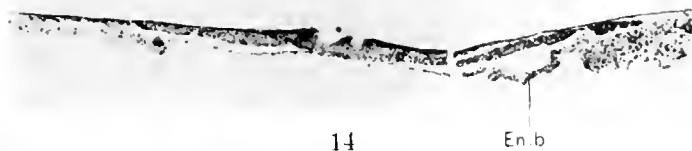
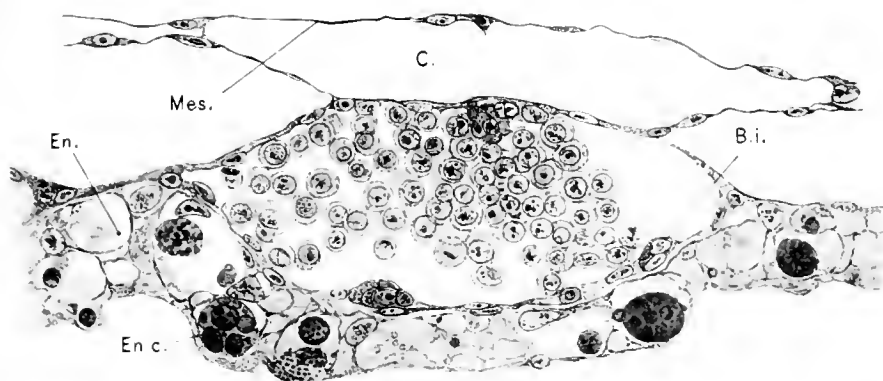


Fig. 13









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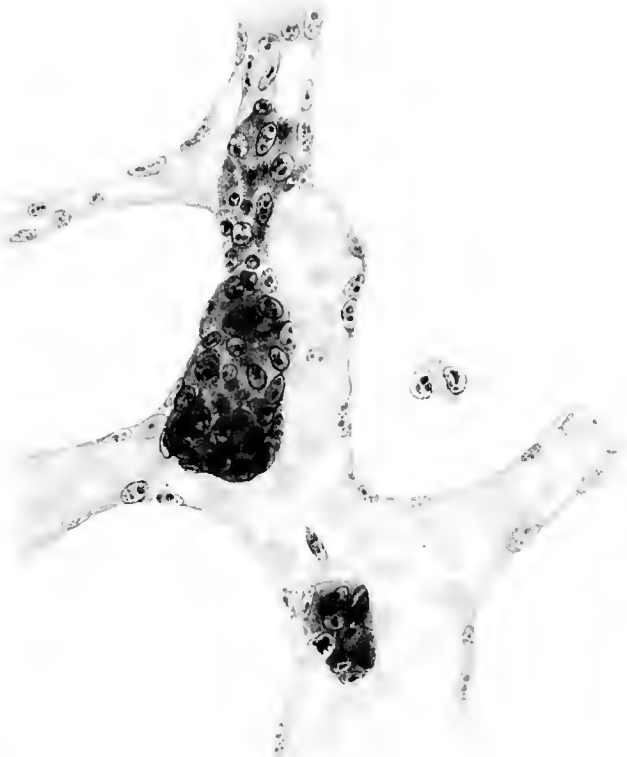


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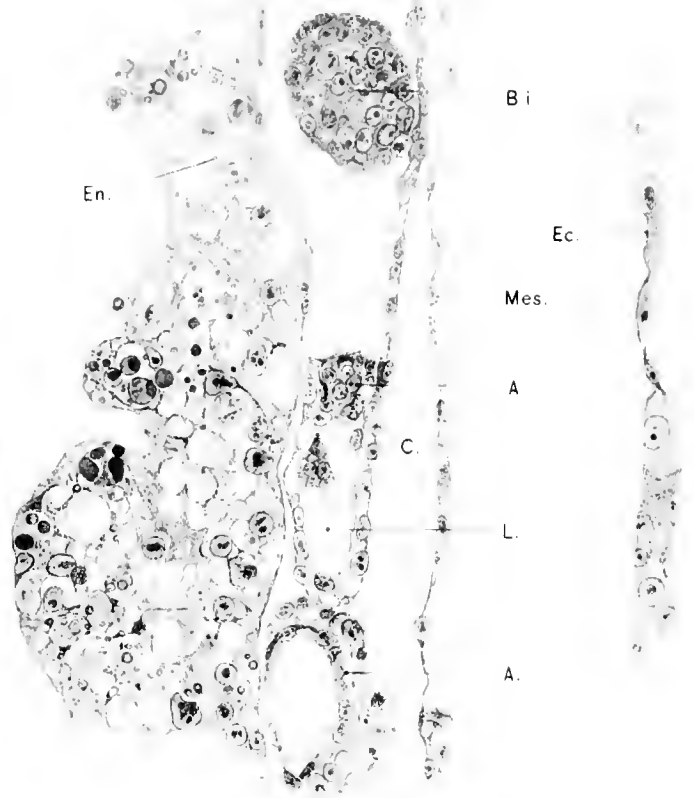


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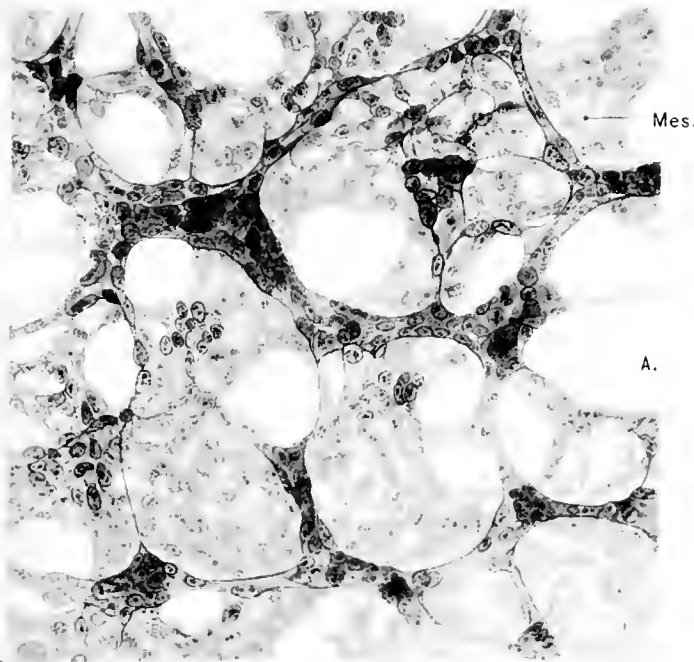




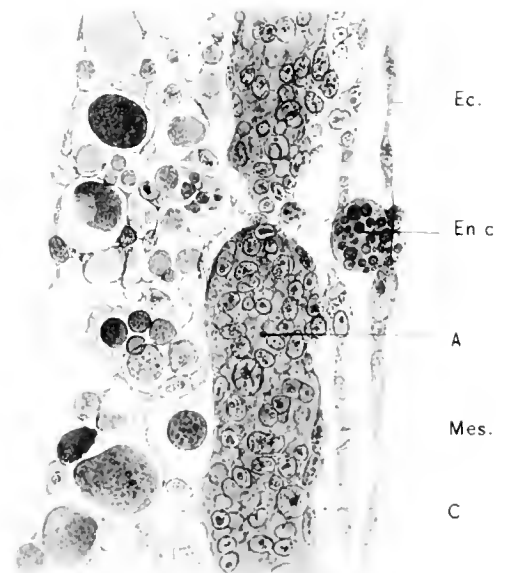
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CONTRIBUTIONS TO EMBRYOLOGY, No. 37.

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NOTES ON THE POSTNATAL GROWTH OF THE HEART,  
KIDNEYS, LIVER, AND SPLEEN IN MAN.

BY ROBERT BENNETT BEAN,  
*Professor of Anatomy in the University of Virginia.*

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With eight text-figures.

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# NOTES ON THE POSTNATAL GROWTH OF THE HEART, KIDNEYS, LIVER, AND SPLEEN IN MAN.

BY ROBERT BENNETT BEAN.

Postnatal growth in man has up to the present time been inadequately treated, but interest is aroused, and we may expect soon to have a much wider knowledge of the subject. The normal weight of the organs is difficult to obtain, and it is not easy even to determine what is normal. Averages may be misleading if there are two or more types in the population. The work of Godin deserves especial consideration in this connection. This author has determined the rate of growth of the linear parts of the body, from birth to maturity, by multiple measurements of individuals, especially those between the ages of 13 and 18 years, on 100 of whom he made measurements every six months over a period of five years. Some of his conclusions are as follows:

The stature is about 50 cm. at birth; in 5 years it has attained an increase of 50 cm., and another 50 cm. is added between the ages of 5 and 15 years. After this the stature increases to the limits of the race, sex, and environment. Individual variations are important and coincident with the onset of puberty. If this is early, whether in girl or boy, the 50 cm. of stature added after 5 years is acquired before the age of 15; if later, after the age of 15. The stature comprises the length of three segments—head, trunk, and extremities. The sitting height (head and trunk) doubles its birth figure at 6 years, and is three times its birth figure in the adult. The lower extremities double at 4 years, triple at 7 years, and quadruple at 15 years. The period elapsing between birth and puberty is 12 to 17 years, 2 years complete the period of puberty, and 3 years are needed after this for maturity.

Godin has compiled certain laws relating to the alternation of growth and rest periods, which parallel the law of alternation in development stated independently by me:

*Godin* (1914): The lower extremities grow rapidly before puberty, the trunk after puberty. The increase in weight is chiefly osseous before puberty, muscular after puberty. The chief growth in stature about puberty occurs during the year preceding puberty; the chief increase in weight about the time of puberty occurs during the period of puberty and the year following. The long bones enlarge in diameter for 6 months and elongate for the next 6 months. The periods of activity and of repose, which succeed each other each six months, are opposite for the two serial bones of the same extremity.

*Bean* (1914): There is one period or more of acceleration, alternating with periods of retardation, in the development of each structural unit or organ of the body. The periods of acceleration in the development of one structure may be synchronous with the periods of retardation in the development of another, and if they are adjacent they may be called complementary structures. Each organ has a critical period when it is developing most rapidly, and when it is probably most susceptible to its environment. The teeth and the

long bones alternate in growth. The growth of the individual teeth alternates from mandible to maxilla or the reverse, and from one tooth to another at some distance from it. The head and thorax alternate in growth, the trunk and extremities, the heart and lungs, the liver and intestines.

The law of alternation in development should be of interest in its relation to the growth of the organs, and it is important to know the relation of the individual to puberty in any study of growth. There is also another law of development that may be illustrated by the growth of the two types in man—the *hyper-phylomorph* and the *meso-phylomorph*. Two types long have been known to physicians as the phthisic and plethoric, or carnivorous and herbivorous; they have also been called the long skeleton and broad skeleton, or narrow back and broad back, but it remains to be determined whether these types are the same respectively as the hyper-phylomorph and meso-phylomorph.

If growth depends upon placental alimentation, as stated by Godin, and if the hyper-phylomorph is precocious and the meso-phylomorph retarded in development, as determined by me (1914), and if the activities of the endocrinous glands influence growth, further studies may reveal some association of these facts, and thus enable us to link up our disconnected studies.

Manouvrier (1902) called attention to the macroskêles (long skeleton) and the brachyskêles (broad skeleton) in different individuals, and Godin (19 0a) has demonstrated that these two types differ from each other in their measurable components more than small individuals differ from tall ones, or men from women. Every transverse diameter is greater in the broad than in the long skeleton. The extremities of the broad skeleton, especially the lower, are relatively short, and of the long skeleton relatively long; but the trunk of the broad skeleton is relatively long, and that of the long skeleton relatively short. The difference in length of the trunk in the two types increases considerably between the ages of 13 and 23 years, and becomes the most striking difference in the linear measurements of the vertical. These differences correspond with those between the hyper-phylomorph and meso-phylomorph. The two types should be discriminated in any study of growth.

The great growth of the extremities and of the teeth (osseous system) before puberty, and the great growth of the trunk after puberty, the latter coincident with the growth of the muscles including the heart, in conjunction with the activity of the sex glands and the maturity of the systems of alimentation, circulation and respiration, undoubtedly are of importance in relation to the growth of the organs.

#### DATA.

Heretofore it has been usual to select for study individuals who died in hospitals, taking only accident cases or those who died suddenly or of acute illness. In this way only the well-nourished would be chosen. It is as inexact to take only the well-nourished as it is to take only the thin. Many factors besides the pathological condition may enter into the state of nourishment. Some individuals are naturally thin, others naturally fat. The type of individual may have an important bearing. In pneumonia the organs are as much above the

average as they are below it in tuberculosis. It may be equally inaccurate to choose only individuals who have died suddenly, because in shock the splanchnic vessels are dilated and the organs may weigh more after death than during life. It may be difficult to devise a method of determining the weight of the organs during life, although Bardeen (1918) has recently succeeded in obtaining what seems to be a satisfactory method of determining the heart weight in the living subject. Any results so far obtained from weighing the organs of the dead are necessarily inadequate and deductions from them should be utilized with caution. It seems best to take all cases, regardless of the cause of death, and study them with as full knowledge as possible concerning their antecedents.

The data for the present study consist of records from the pathological department of the Charity Hospital, New Orleans, and from the pathological department of the Johns Hopkins Hospital, Baltimore. Some of those from the Charity Hospital were copied by Dr. Wilmer Baker, and all of them represent the submerged tenth of the semi-tropics; whereas those from the Johns Hopkins Hospital represent an average hospital population of a large, modern city. No attempt has been made to select only normal organs, but where an organ is obviously diseased, so as to materially affect its weight, it is discarded.

#### HEART.

The weights of the organs were treated as Mall (1918) treated the crown-rump length of embryos; *i. e.*, each individual is represented on a chart one meter square, with the weight as the ordinate and the age as the abscissa. The growth of the heart in both sexes and both races (white and negro) appears to be divided into three periods of rapid growth, alternating with three periods of slow growth; the first period of rapid growth is between 3 and 9 months, the second from 2 to 8 years, and the third from 14 years to maturity. There is no relative decrease in the increment of growth in each consecutive period from the first to the third. The rate of growth during any period is based upon the size of the heart at the beginning of that period of growth.

The heart of the female is smaller than that of the male except at about the tenth year, when it is larger. This corresponds to the time at which the stature of the female is greater than that of the male. Heart weight, size of individual, and physical activity are synchronous. For the first few months after birth the child is not very active, and during this period the growth of the heart is slow. From 6 to 9 months of age the child becomes more active, the growth in stature increases, and the heart enlarges rapidly. Then follows a less active period, when the child is learning to stand and walk, and during this time the heart is proportionately inactive. After the child has learned to walk and run, a period of great activity ensues and the stature and heart weight increase rapidly up to the age of 8 years. After this there is a slowing down of this increase, followed by an acceleration, in girls at about the age of 10 years, in boys somewhat later; at this age, therefore, girls surpass the boys in stature and heart weight, to be again

passed by the boys at about the age of 14 years, after which there is an increasing difference until maturity.

The weight of the heart varies more with the size of the individual than with age, sex, or race, and it may be taken as an index of the size and activity of the individual, other things being equal. The weights given by Bardeen, Vierordt, Kress and others were placed on charts similar to those of my own, and similar differences were found, although the weights given by these authors were greater than my own, due to a difference in the selection of material. If these charts are analyzed it will be found that at almost every age, in both sexes and both races, there is a group of small hearts and a group of large hearts. In the early years there is no distinct line of demarcation for the two groups, no zone where there is complete absence of the heart weights; but after the age of 14 years such a zone appears, and when the age of 17 years is reached there are no heart weights between 225 and 250 grams in my records.

The cause of death was equally divided between acute and chronic disease in both large and small hearts during the early ages; but at the age of 17 years there are more large hearts among the acute cases and more small ones among the chronic cases. The stature does not appear to alter materially the heart weight, since in acute cases with large hearts the stature was about the same as in chronic cases with small hearts; while in chronic cases with large hearts the stature was 6 cm. less than that of acute cases with small hearts.

The type of the individual seems to affect the heart weight, as, even after discarding cases supposedly influenced by disease, there still remain two groups, large and small. The same is true when only accident cases are tabulated. The largest hearts are found in cases of pneumonia, and the smallest in cancer and tuberculosis. Not all of the large hearts, however, are found in pneumonia cases, nor do all the small hearts occur among the wasting diseases. In the former it might be inferred that the physique, the nourishment, or the disease has caused the excessive size, but there seems to be little evidence that pneumonia causes any considerable increase in the weight of the heart; therefore, the large size of the organ may be attributed to the type of individual. In wasting diseases there is loss of weight in the heart, but a part of the excessive smallness may also be attributed to the type. The meso-phylomorph is susceptible to cardio-vascular, renal, and acute diseases, especially pneumonia; the hyper-phylomorph to nervous, alimentary, and wasting diseases, as already pointed out by me (1912).

An average may be nothing more than a mean between two types, and the individual may be of greater value in a study of this kind than a mass of averages.

#### LIVER.

At 2 months the liver weighs little more than at birth, after which there appear to be three periods of rapid growth; the first from 3 to 12 months, the second from 2 to 8 years, and the third from 14 years to maturity. There is a decrease in the increment of growth in each consecutive period from the first to the third. The

growth of the liver from 3 to 12 months is greater than that of the heart, and less than that of the kidneys.

The liver of the female is smaller than that of the male except at about the age of 10 years, when it exceeds the latter in size. This corresponds to stature and heart weight and is evidence of precocity in the female. The liver of the negro is smaller than that of the white up to the age of 14 years; after that age the liver of the negro female seems to exceed in weight that of the white female. However, more data are necessary to confirm this.

The weight of the liver at all ages falls naturally into two groups—large and small. This difference in size is especially noticeable after the age of 14 years, at which time the weight may be subdivided into four groups. This is true also for the other organs, but with them it is not so distinct. The extremely small livers are from individuals who have died of such wasting diseases as tuberculosis, the extremely large ones almost exclusively from those who have died of pneumonia. This organ, more than the others, seems to be affected by such diseases. The extremes of weight in tuberculosis are 500 and 1,200 grams, while in pneumonia they are 1,800 and 2,800 grams.

#### KIDNEYS.

The two kidneys are treated as one and charted as were the heart and liver. The initial increase in growth, *i. e.*, 3 to 12 months, is greater and more rapid in the kidneys than in the other organs, and their maturity seems to be reached earlier, or at about the age of 15 years. Other than this the kidneys have corresponding periods of rapid and slow growth, which fall at about the same time as those of the other organs.

The kidneys are smaller in the female than in the male except at about the age of 4 years, when they are slightly larger. Their weight is at all times greater in the negro female than in the white female, and inversely greater in the white male than in the negro male. Only after the age of 14 years does the weight fall, to any appreciable extent, into the two groups; and even then these groups are not so distinct as in other organs.

#### SPLEEN.

The growth of the spleen is similar to that of the other organs—rapid during the second 6 months of life, from 2 to 8 years, and from 14 to 18 years, with intervening periods of slow growth. However, a few irregularities may be worthy of note. A greater number of large spleens, for example, are observed at 2 weeks after birth, at 7 months, and at about 7 and 10 years; and more small spleens about the ages of 6 and 13 years. In the male the weight of the spleen remains practically the same from the sixth month to 2 years. In males the weight is always greater than in females, but there is little difference between the sexes at the ages of 8 and 12 years. It is likewise greater in whites than in negroes, at all ages and in both sexes. This seems to be a distinct racial difference. After the age of 14 years, but not before, the spleen weight may be grouped at about 100 grams and 200 grams, thus representing two types.

## SUMMARY.

Each organ has a period of rapid growth soon after birth, a second period from 2 to 8 years, and a third period about puberty. Each of these periods alternates with one of slow growth. As growth proceeds each organ becomes either large or small, depending upon the type of individual. There are changes in size due to the state of nourishment and to pathological conditions; race and sex also influence the size of the organs. The most characteristic racial difference is found in the spleen, which is larger in the white than in the negro.

The rate of growth is determined from the weight of the organ preceding the period of growth under consideration; that for the first 2 years is based upon the weight of the organ at birth; the other periods begin at 2, 10, and 18 years respectively.

The growth of the heart is slower during the first two years than is that of the spleen, liver, or kidneys; but after that time, until maturity is reached, the rapidity of its growth is relatively greater than that of the other organs. During the periods after the second year the kidneys grow relatively less than the spleen, and the spleen relatively less than the liver.

In conclusion, attention is called to the great importance of collecting and carefully studying material about which the following data may be ascertained: Age, sex, race, type, relation to puberty, antecedent habits, heredity, condition of nourishment, pathological condition, and the actual cause and condition of death. The type and the relation to puberty are especially important, and it may be helpful to obtain the largest possible number of cases of instantaneous death. This will necessitate, of course, the cooperation of a large number of observers at different places and over considerable periods of time. The interest that has already quickened into activity, however, bids fair to result in better records for the future.

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- |   |   |
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|---|---|



## TABLES.

The first four tables given here are from records in the Pathological Department of the Johns Hopkins Hospital. The weights are given in grams and are omitted when they are obviously altered by the pathological condition. No pathological records are given, but each record was studied separately in order to determine which organs to eliminate and which to retain, as well as for other purposes. The last four tables are from records in the Pathological Department of the Charity Hospital, New Orleans, Louisiana, and have been treated the same as the other records. For the use of these data I am indebted to the courtesy of Dr. W. G. MacCallum, of the Johns Hopkins Medical School, and Dr. C. W. Duval, of the New Orleans Charity Hospital.

	White female.	White male.	Negro female.	Negro male.	Totals.
Johns Hopkins Hospital records.	203	255	246	264	968
Charity Hospital records.....	52	81	115	159	307
Totals.....	255	336	361	423	1375

TABLE 1.—*Johns Hopkins Hospital, White Female Children.*

J. H. H. No.	Age.	Heart weight.	Spleen weight.	Liver weight.	Kidney weight.	J. H. H. No.	Age.	Heart weight.	Spleen weight.	Liver weight.	Kidney weight.
5231	11 months..	25	13	210	L. 37	4971	1 year....	25	200		
3953	11 months..	30	20	270	70	4986	11 months..	26	18	200	R. 38
3964	5 months..	20	10	100	40	4807	3.5 months	30	18	175	26
3967	4 months..	15	10	85	20	4821	11 months..	25	240	52	
3977	1 year....	20	10	400	25	4822	1 year....	39	25	320	67
4615	10 months..	55	40	340	55	4824	3 months..	20	13	175	27
4625	10 months..	20	25	405		4826	16 months..	42	300	57	
4628	5 minutes..	4	3.5	33	R. 2.5	4829	2.5 years..	50	45	400	L. 45
4645	3 weeks....	21	5		R. 9	4854	3 years....	38	420	82	
4652	1 year....	20	10	250		4858	10.5 years..	130	95	850	195
4658	1 year....	23	33	180	R. 26	5376	9 years....	150	145	940	
4673	1 month....	23	17.5	180	27.5	4859	2.5 years..	56	39	380	51
4689	5 hours....				18	4860	2.25 years..	61	22	320	66
4692	3 months..	22	22	230	R. 18	4866	11 years....	115	55	600	139
4699	Still-born..				L. 8.5	4869	5 years....	70	39	485	72
4508	3 years....		50	460	60	4872	2.66 years..	55	34	253	68
4526	1 year....	27	23	285	38	4875	2 years....	52	39	430	76
4572	1 month....	18	5	180		4879	1.75 years..	60	40	440	89
4539	3 years....	68	105	340	L. 5.2	4882	6 months..	31	17	243	36.5
4589	Still-born..	23	16	205	29.5	4708	Still-born..	24	13.5	150	28
4590	13 months..		18		R. 24	4727	3 years....	90	80	470	115
4591	8 months..	30	20	310	53	4748	10 months..	40	23	70	80
4307	6 years....	85	62	645	110	4757	1 month....	14.5	7	110	L. 7.5
4364	2.5 years..	50	50	500		4777	16 months..	37	17	308	73
4372	13 months..		17	210		4780	9 years....	145	80	690	L. 95
4375	6 months..	40	20	290		4794	4 months..	17.5	11		24
4381	5 days....	40				4797	16 months..	26	11	250	52
4393	2 years....	50	40	380	90	5010	2.5 hours..	7	2	33	R. 5
4399	12 years....		90	1200	200	5016	Still-born..	9	2	40	R. 6
4208	8 months..	10	10	100	40	5017	3 days....	18	8	160	12
4219	7 weeks....	22	50	180	40	5023	4 months..	29	18	210	35
4230	13 months..	25	18	90	70	5037	Still-born..	4	2	38	7
4256	14 months..	25	100	420	42	5040	Still-born..	12.5	6	80	R. 11
4261	4.5 hours..	10		70	20	5043	16 months..	100	22	300	R. 33
4903	8 months..	60	25	425	32	5064	9 months..	36	30	300	R. 65
4913	19 months..	50	32	389	88	5084	2 years....	50		400	R. 40
4924	5 years....		75		129	5093	Still-born..		1	45	7
4945	2 years....	60	20	390	72	5102	2 months..	13	5	100	R. 30
4951	18 months..	39	37	355	64	5115	15 months..	50	40	400	40
4958	3.5 years..	90	40	450	78	5117	10 months..	27	8	130	16

TABLE 1.—*Johns Hopkins Hospital, White Female Children—Continued.*

J. H. H. No.	Age.	Heart weight.	Spleen weight.	Liver weight.	Kidney weight.	J. H. H. No.	Age.	Heart weight.	Spleen weight.	Liver weight.	Kidney weight.
5119	10 years...	160	120	1000	R. 100	1963	17 years...	250	140	1370	.....
5120	5 years...	.....	50	640	R. 85	1807	5 years...	110	100	800	200
5137	1 year...	50	40	340	40	1819	10 years...	150	100	850	250
5141	7 months...	27	17.5	210	R. 24	1890	17 years...	220	.....	1600	390
5167	4 months...	22	22	215	37	1639	0 .....	.....	9	132	26
5179	Still-born...	8.75	6	63	R. 12	1513	16 years...	170	280	1600	L. 135
5201	3 months...	.....	.....	180	R. 17	1518	10 years...	.....	125	1050	280
5219	6 months...	24	17	220	R. 24	1637	20 years...	230	100	1150	.....
5484	13 months...	30	12	280	46	1508	21 years...	260	240	1630	345
5222	1 year...	38	23	305	50	4139	New-born...	25	20	110	.....
5512	15 months...	.....	45	360	.....	5513	14 years...	240	110	1660	R. 200
5412	17 months...	54	.....	.....	R. 40	4151	3 years...	60	40	360	90
5336	18 months...	31.5	22	230	R. 28	3867	14 years...	.....	275	1500	.....
5497	1 year...	40	15	300	R. 40	3701	Still-born...	20	15	.....	.....
5474	2 years...	60	52	370	R. 42	3704	17 years...	200	110	1250	350
5533	2 years...	45	50	400	R. 35	3776	12 years...	150	.....	1300	300
5360	3 years...	90	70	650	105	3518	5 weeks...	40	15	170	35
5546	3.5 years...	.....	20	900	L. 45	3526	16 years...	280	210	1800	440
5264	4 years...	.....	.....	.....	L. 80	3544	18 months...	.....	50	500	115
5424	4 years...	80	45	.....	L. 49	3534	7 years...	120	50	650	120
5487	5 years...	60	40	500	80	3426	16 years...	200	200	1100	250
5459	9 years...	170	70	1020	R. 100	3431	Still-born...	40	30	160	.....
1487	20 years...	.....	.....	1600	350	3443	15 months...	70	50	320	100
1597	19 years...	170	110	.....	280	3444	1 year...	70	50	350	100
1497	20 years...	150	200	1750	.....	3455	18 months...	120	.....	450	.....
2157	20 years...	210	200	1590	340	3309	12 years...	130	.....	900	200
3402	18 years...	.....	170	1600	360	3246	14 years...	200	80	1250	.....
3741	19 years...	370	220	2000	390	3285	6 years...	90	42	.....	150
3884	22 years...	.....	100	1500	310	3118	15 years...	180	.....	1500	L. 175
3936	20 years...	.....	180	1100	240	3129	13 years...	.....	145	1340	.....
3939	16 years...	.....	150	1550	.....	3175	13 years...	.....	60	850	150
4135	19 years...	215	100	1045	250	3026	19 years...	.....	90	1750	430
4212	20 years...	.....	100	1900	280	3067	3 years...	65	35	500	.....
4242	18 years...	300	190	1770	350	3098	9 years...	.....	50	1080	150
4489	20 years...	200	250	1625	R. 135	2925	4.5 years...	70	50	850	170
4349	20 years...	200	90	1500	370	2855	13 years...	125	30	830	150
1586	10 years...	120	.....	820	200	2717	14 months...	.....	30	350	100
1474	14 years...	.....	100	1550	L. 150	2738	Still-born...	20	15	180	.....
1479	10 months...	40	30	365	R. 30	4411	10 months...	35.5	23.5	315	60
1324	2 years...	.....	50	550	.....	4415	14 months...	.....	17	.....	.....
1370	6 years...	.....	30	680	145	4421	7 years...	.....	60	540	180
1393	13 days...	20	10	150	30	4430	1 month...	.....	10	140	.....
1235	13 days...	20	15	70	22	4431	19 months...	48	40	375	64
1249	16 years...	250	220	1210	.....	4448	10 months...	.....	3	180	30
2758	9 years...	.....	70	950	200	4449	1 day...	21	9	130	35
2779	12 years...	90	50	1200	150	4452	2 months...	11.5	6	100	13
2539	16 months...	.....	50	360	.....	4454	18 years...	130	180	1400	250
2592	4 days...	.....	5	50	20	4459	3 months...	15	9	130	R. 25
2420	12 years...	140	220	950	.....	4460	3 months...	28	12	160	R. 43
2429	2 years...	50	50	.....	150	4472	14 months...	30	.....	300	R. 30
2438	12 years...	.....	.....	1240	190	4285	8 months...	23	15	170	40
2495	2 years...	50	50	400	75	4035	9 months...	.....	16	140	60
2309	15 years...	.....	160	1580	340	4070	1 year...	35	15	255	65
2341	8 years...	.....	225	1200	300	4076	4.5 years...	.....	.....	.....	L. 60
2352	3.5 months...	20	65	250	90	4082	8 months...	35	10	170	35
2103	18 years...	250	100	.....	250	4098	15 months...	40	45	250	60
2133	10 days...	23	8	130	22	5221	3 months...	26	14	165	43
2143	10 days...	35	20	120	20	5222	1 year...	38	23	305	R. 23
2213	8 years...	75	60	710	.....	5224	4 months...	27	12	210	27
2289	6 years...	70	50	390	120	5229	Still-born...	3.2	1.2	22.5	4.5
1911	15 years...	90	60	800	200	5230	Still-born...	6	.75	18	3.75
1916	4 hours...	28	7	135	21						

TABLE 2.—*Johns Hopkins Hospital, White Male Children.*

J. H. H. No.	Age.	Heart weight.	Spleen weight.	Liver weight.	Kidney weight.	J. H. H. No.	Age.	Heart weight.	Spleen weight.	Liver weight.	Kidney weight.
1873	10 years...	120	170	970	270	3004	15 years...	200	1020	300	
1768	14 years...	170	.....	1500	350	3018	10 months...	20	250	50	
1796	14 years...	200	.....	.....	300	3061	7 years...	110	1000	250	
1631	17 years...	.....	70	1020	.....	3080	16 years...	250	160	1700	.....
1690	4 years...	80	60	700	150	3087	17 years...	210	.....	2270	340
1529	18 years...	.....	200	1200	270	2934	9 years...	120	50	1000	200
1538	14 years...	200	.....	.....	330	2844	19 years...	300	.....	1820	400
1539	9 days...	.....	20	70	L. 12	2845	2.5 years...	70	20	500	70
1411	19 years...	280	.....	1900	350	2872	5 years...	95	50	700	.....
1435	18 years...	.....	.....	2000	R. 250	4400	5 months...	.....	24	270	60
1496	8 years...	110	60	900	L. 90	4405	1.5 hours...	22	11	155	29
1319	4 years...	.....	80	560	.....	4428	2 months...	.....	10	140	32
1364	13 years...	150	70	700	260	4433	2 years...	.....	.....	440	.....
1214	4 years...	70	90	480	100	4435	6 years...	70	100	510	210
1170	10 years...	120	75	700	170	4438	4 years...	65	40	560	200
2897	14 months...	50	40	320	50	4440	4 years...	.....	50	750	200
2740	6 years...	100	50	1100	200	4441	5 months...	28	17	167	50
2747	6 years...	120	100	700	170	4445	17 years...	.....	198	2000	375
2752	8 years...	75	60	820	150	4466	2 days...	8	2.5	38	R. 5
2768	18 years...	270	270	2600	.....	4467	New-born...	24	.....	.....	R. 7
2769	18 years...	250	.....	1550	.....	4480	2 years...	70	50	425	90
2589	6 years...	85	.....	530	130	4499	13 months...	26	20	225	R. 30
2433	Still-born...	15	10	90	20	4118	1 year...	.....	50	.....	.....
2462	20 days...	20	.....	125	.....	4149	38 months...	.....	30	450	90
2470	17 months...	50	.....	600	.....	4155	3 months...	40	25	230	65
2325	17 months...	60	.....	600	140	4177	3 months...	20	10	110	L. 25
2363	14 years...	210	110	1000	380	4181	9 months...	22	12	210	.....
2125	5.16 months	20	14	155	40	4182	22 months...	50	.....	520	100
2141	7 years...	90	50	825	180	3828	16 years...	.....	220	1870	320
2157	15 years...	90	70	800	L. 90	3893	9 months...	40	.....	300	L. 40
2168	18 years...	250	.....	1800	.....	3709	17 days...	20	10	150	50
2171	3 months...	25	15	229	45	3717	4 years...	80	.....	480	.....
2172	5 years...	.....	50	620	.....	3721	13 months...	.....	20	240	90
2204	18 years...	.....	150	1800	350	3724	16 years...	.....	.....	1200	330
2258	18 years...	250	100	2500	350	3747	15 years...	200	110	1950	300
2284	10 years...	150	50	800	150	3781	3 years...	60	.....	450	120
2027	14 years...	120	70	640	180	3794	3 years...	70	50	.....	120
2046	16 years...	.....	150	1350	200	3605	6 years...	75	45	650	140
2066	18 years...	200	.....	1550	300	3670	6 years...	70	40	450	.....
1910	7 years...	115	95	870	190	4670	3 months...	.....	14	190	39
1915	1 year...	30	50	200	100	4525	6 days...	13	6	.....	L. 10
1918	3 years...	70	70	700	120	4541	5 days...	.....	10	100	16
1945	5 years...	80	.....	950	.....	4553	6 years...	98	50	600	110
1966	7 years...	.....	100	850	.....	4577	Still-born...	.....	3	.....	10.5
1981	12 days...	30	40	140	50	4579	5.5 years...	82	40	480	.....
1993	17 years...	250	80	1650	300	4581	4 years...	90	20	450	130
1808	10 years...	100	60	770	.....	4585	1 year...	50	30	300	84
1842	17 years...	.....	.....	1500	L. 150	4587	8.5 hours...	.....	16	205	29.5
4079	20 months...	52	35	580	.....	4592	9 weeks...	23	15	170	39
4083	16 days...	10	5	120	L. 40	4314	2 years...	50	25	580	110
4088	6 years...	85	170	590	110	4316	2.5 years...	90	60	450	100
4092	1 year...	40	32	330	95	4353	7 years...	.....	45	970	225
4094	2.5 years...	62	42	575	150	4367	13 months...	55	25	355	65
3685	16 years...	270	150	1500	.....	4371	1 month...	5	3	50	.....
3531	12 years...	.....	55	840	.....	4388	5 months...	.....	40	300	.....
3586	9 years...	120	120	720	170	4215	14 months...	25	.....	.....	60
3424	15 years...	.....	160	1260	250	4225	1.5 years...	.....	.....	400?	110
3479	14 years...	150	.....	980	220	4226	3 months...	20	10	130	35
3481	Still-born...	20	.....	70	.....	4283	1 month...	25	.....	.....	45
3493	2 years...	25	20	250	70	4288	30 minutes...	35	.....	.....	50
3497	Still-born...	20	20	150	40	4297	12 days...	.....	12	158	45
3308	7 years...	90	.....	520	.....	4005	2 months...	15	5	80	22
3361	2 months...	20	.....	.....	.....	4023	13 weeks...	25	.....	125	25
3371	10 years...	.....	100	1200	220	4044	4.5 years...	80	50	600	125
3374	4 years...	70	40	640	.....	4065	6 days...	35	.....	140	.....
3383	8 months...	40	40	270	70	4066	10 months...	40	25	270?	60
3235	7 years...	60	50	370	100	4078	4 months...	20	10	.....	.....
3239	8 years...	150	60	1050	.....	4855	11 months...	18	45	460	67
3245	19 months...	50	20	300	65	4856	Still-born...	8	1.5	40	L. 5
3172	9 years...	120	100	850	170	4862	22 months...	39	16	240	L. 41
3003	29 months...	80	40	500	.....	4865	4.5 years...	61	44	500	90

J. H. H. No.	Age.	Heart weight.	Spleen weight.	Liver weight.	Kidney weight.	J. H. H. No.	Age.	Heart weight.	Spleen weight.	Liver weight.	Kidney weight.
4867	29 months..	72	28	400	70	4848	11 months..	39	47	365	72
4868	10 months..	42	19	390	59	4849	1 year.....	20	26	440	R. 28.5
4873	9 weeks....	19	25	140	40	5005	5 months..	9	140	140	R. 21
4874	4.75 years..	92	37	589	98	5007	8 months..	40	70	200	20
4877	3 years.....	55	35	.....	50	5031	4 years....	90	60	730	R. 60
4878	9 months..	55	29	448	93	5054	3 weeks....	30	16.5	120	R. 13.5
4881	15 months..	31	15	420	61	5058	3 months..	12.5	9	.....	R. 18
4887	5 months..	27	19	257	63	5088	15 years..	180	180	.....	R. 120
4891	3.5 years..	68	27	370	80	5090	4.5 years..	80	35	500	R. 35
4892	9 years....	125	65	760	190	5098	4 months..	21	16	.....	R. 20
4894	16 years..	235	.....	1500	290	5154	Still-born..	8	6	68	21
4895	10 months..	23	20	400	.....	5158	2 months..	60	6	140	.....
4899	8 months..	.....	20	250	60	5162	5 years....	100	60	740	125
4706	1 hour.....	6.5	2	40	R. 5	5165	4 years....	70	45	.....	84
4720	9 months..	45	20	270	80	5190	5 years....	100	36	480	60
4725	11 months..	80	75	410	95	5194	Still-born..	16	4.5	70	R. 15
4730	2 days.....	8	3.5	85	12.5	5220	9 months..	24	17	220	R. 24
4738	5 months..	24	12	320	.....	5226	8 years....	118	72	840	158
4740	4 months..	47	7	.....	R. 18	5232	5.5 months	14	6	90	.....
4747	6 months..	21	14	120	R. 17	3962	8 days.....	.....	12	140	R. 21
4763	Still-born..	14	4.5	100	22	3966	17 months..	40	25	320	L. 55
4772	6 months..	28	15	295	45	3974	4 years....	50	.....	450	100
4781	13 months..	.....	20	.....	53	3975	10 years..	150	110	980	260
4784	3 months..	19	13	123	40	3984	16 months..	50	65	350	110
4603	5 days.....	.....	6	.....	20	3986	7 days....	15	5	100	50
4611	7 months..	75	20	300	80	3990	4 months..	20	10	130	32
4639	Still-born..	.....	4	.....	14	3809	3 months..	35	20	240	37
4653	Still-born..	.....	3.5	.....	14.5	1106	20 years..	250	110	1330	145
4654	1 year.....	60	25	350	74	1493	21 years..	200	120	1600	L. 130
4904	6 weeks....	24	16	160	40	1397	20 years..	370	.....	1900	L. 108
4908	4 months..	12	5	68	21	1432	20 years..	.....	.....	1800	L. 150
4915	9 months..	35	20	300	50	1647	20 years..	280	.....	2910	.....
4916	2 years....	56	45	417	73	3100	21 years..	.....	240	1970	280
4925	2.25 years.	62	40	430	103	3436	21 years..	240	.....	2200	360
4931	1 year.....	42	45	392	64	3458	19 years..	.....	250	1520	.....
4932	3 months..	20	7	130	35	3449	21 years..	420	.....	1940	420
4936	13 years..	140	80	980	200	3733	21 years..	.....	170	.....	L. 120
4942	22 months..	.....	20	490	.....	3796	18 years..	185	.....	1570	330
4961	14 months..	45	40	400	80	3611	20 years..	240	.....	1940	.....
4969	Still-born..	16	6	75	L. 11	3869	19 years..	.....	100	1000	L. 125

TABLE 3.—*Johns Hopkins Hospital, Colored Female Children.*

J. H. H. No.	Age.	Heart weight.	Spleen weight.	Liver weight.	Kidney weight.	J. H. H. No.	Age.	Heart weight.	Spleen weight.	Liver weight.	Kidney weight.
2446	3 years...	70	40	420	70	4434	18 months..	78	60	450	150
2457	16 years...	200	220	.....	320	4446	16 years...	200	150	1625	250
2472	2 years...	.....	.....	525	100	4453	3 months..	40	7.5	200	70
2474	2 months..	.....	20	170	45	4462	10 days....	.....	12	330	.....
2318	14 years...	220	80	1150	170	4465	2.5 months	21.5	8.5	187	R. 27
2107	14 years...	150	130	1200	.....	4483	Still-born..	13	7.5	60	18
2138	14 years...	180	150	1300	300	4485	5 months..	.....	7	120	R. 15
2153	6 years...	70	40	400	140	4491	10 months..	34	11	180	R. 37
2160	11 months..	25	15	150	40	4488	15 days....	12	8.5	80	18
2166	14 months..	20	20	200	10	4318	46 months..	50	25	400	120
2180	3 years...	40	30	270	.....	4374	4 years...	50	45	500	110
2190	18 years...	240	120	1740	260	4384	14 years...	210	100	1090	180
2266	4 years...	100	40	540	140	4394	15 years...	180	100	1120	.....
2023	7 months..	30	20	.....	30	4240	3 years...	.....	.....	400	100
2093	10 years...	150	70	955	220	4014	4 months..	10	45	100	L. 10
1976	19 years...	200	140	1350	R. 120	4021	9 months..	25	70	310	80
1800	3 years...	50	.....	370	70	4040	.....	65	120	640	140
1818	18 years...	170	170	1470	280	4061	9 years...	.....	45	650	210
1837	14 years...	150	125	1675	330	4084	6 weeks...	25	22	180	55
1853	13 years...	80	60	1050	250	4095	13 months..	45	.....	265	90
1893	8 years...	100	80	940	.....	3908	11 months..	20	.....	.....	.....
1767	18 years...	.....	.....	.....	320	3931	9 months..	40	40	275	L. 30
1655	10 years...	130	.....	1020	240	4782	1 year....	32	8	180	50
1661	18 years...	250	.....	1450	.....	4789	8 years...	100	35	670	155
1694	8 years...	140	40	1000	190	4604	32 hours...	13	6	.....	12
1559	14 years...	230	100	1800	.....	4616	1.5 years...	33	25	215	69
1429	19 years...	280	160	1600	350	4617	2 years...	60	.....	.....	105
1311	14 months..	45	40	350	.....	4624	2 years...	50	14	200	46
1245	17 years...	.....	130	1400	460	4629	2 months..	42	15	125	48
1251	19 years...	230	80	1400	R. 150	4633	Still-born..	13.5	2	35	12.5
1252	19 years...	250	145	.....	270	4638	3 years...	80	15	420	120
1257	17 years...	170	150	1580	220	4640	10 years...	120	40	730	185
1135	13 years...	120	40	790	180	4661	5 months..	26	18	140	32
4101	2 years...	50	.....	360	L. 50	4665	8 months..	55	19	260	R. 24
4113	6 months..	30	50	310	60	4672	Still-born..	.....	22	215	L. 26
4125	4 days....	20	5	110	.....	4678	12 hours...	.....	9	130	L. 8
4130	6 months..	40	25	160	80	4685	4 months..	.....	25	300	R. 20
4136	7 months..	10	5	60	25	4690	10 months..	27.5	8.5	175	L. 32
4144	Still-born..	15	10	145	20	4697	2 years...	55	30	365	105
4147	4 months..	.....	.....	130	20	4509	Still-born..	.....	15	140	24
4160	1 year....	40	30	230	70	4535	9 days....	.....	7	90	8
4162	3 months..	40	20	.....	70	4552	Still-born..	10	10.5	54.5	9
4166	4 months..	15	5	100	20	4557	4 months..	35	60	250	R. 30
4174	14 years...	80	25	540	120	4559	Still-born..	12	13	80	16
4175	3 weeks...	5	3	80	20	4562	New-born..	15	7	90	23.5
4192	18 months..	45	25	360	85	4563	18 years...	275	170	1550	240
3737	15 years...	170	.....	1120	270	4571	1 day....	8.5	3.5	49.5	13
3762	17 years...	210	120	1660	320	4574	New-born..	.....	55	80	32
3792	3.5 months	35	25	215	50	4576	17 years...	300	120	1400	250
3609	4.5 years...	70	35	400	.....	4578	13 years...	280	95	1020	230
3637	17 years...	170	75	1460	300	4597	3 hours...	21.5	18.5	130	25.5
3505	26 days...	20	10	150	40	4301	Still-born..	23	12	125	25
3522	7 years...	70	90	690	120	4306	40 minutes.	.....	20	240	45.
3556	9 years...	145	70	930	220	4910	Still-born..	13.5	10.2	72	28
3560	Still-born..	30	20	125	45	4929	5 days....	10	.....	.....	8.9
3566	3 years...	.....	20	400	70	4937	Still-born..	11	10	37	L. 6
3578	14 years...	150	100	1350	250	4938	Still-born..	16	16	120	15
2719	6 weeks...	20	20	190	40	4949	2 years...	.....	25	350	80
2739	18 years...	300	100	1400	350	4952	Still-born..	23	14	115	60
2753	14 years...	240	.....	1700	250	4991	Still-born..	18	4	100	18
2642	17 years...	.....	170	.....	.....	4960	2 years...	102	28	450	85
2697	14 years...	200	150	1750	300	4992	Still-born..	9	2	43	9
2566	18 years...	250	150	1200	360	4800	3 days....	20	6	150	35
2572	5 months..	40	40	160	.....	4803	13 years...	170	50	980	171
2430	19 months..	20	70	300	70	4806	4 months..	26	18	50	10.2
2440	10 years...	200	.....	1300	.....	4813	4 months..	16	10	145	40
4406	19 days....	.....	7	90	14	4819	2 years...	50	30	430	100
4414	2 weeks...	14	19	120	45	4828	2 years...	52	17	310	64
4420	4 weeks...	25	.....	.....	20	4833	2 years...	50	22	340	56
4422	14 months..	28	17	420	80	4838	New-born..	17	6	75	36
4425	11 days....	18.5	8	140	48	4861	1 year....	45	45	360	100

TABLE 3.—*Johns Hopkins Hospital, Colored Female Children—Continued.*

J. H. H. No.	Age.	Heart weight.	Spleen weight.	Liver weight.	Kidney weight.	J. H. H. No.	Age.	Heart weight.	Spleen weight.	Liver weight.	Kidney weight.
4876	4 years...	68	28	400	80	3302	13 years...	170	50	750	200
4880	Still-born..	10	30	61	R. 8	3301	4 years...	70	.....	.....	150
4885	3 years...	58	30	351	84	3366	10 years...	120	60	900	200
4893	5 months...	30	15	235	42	3209	7 years...	70	25	370	100
4897	10 years...	.....	50	760	210	3238	17 years...	250	.....	1750	.....
4898	20 months...	54	31	260	59	3280	18 years...	250	170	2120	390
4710	6 days...	19	11.5	110	31	3168	6 years...	70	50	.....	120
4712	26 days...	9	5	50	16.5	3008	17 years...	190	80	1570	270
4713	Still-born..	14	3	40	16	3033	13 years...	160	110	1020	180
4737	8 months...	34	16	240	49	2986	18 years...	260	200	1680	.....
4743	Still-born..	26.5	3.5	190	35	2813	20 years...	220	150	2050	520
5009	Still-born..	35	13	110	R. 26	2737	18 years...	290	130	1920	270
5003	5 years...	50	80	930	R. 60	1126	18 years...	210	95	1500	R. 125
5029	11 years...	133	52	920	R. 90	1167	16 years...	150	90	1450	250
5036	11 days...	16	28	85	R. 9	1179	18 years...	350	.....	1700	370
5044	2 months...	16	13	.....	R. 16	1361	20 years...	200	170	.....	250
5045	3 years...	90	50	.....	R. 75	1405	20 years...	200	.....	1500	375
5067	Still-born..	12	7	170	R. 12	1439	20 years...	220	.....	1680	355
5072	10 years...	185	76	1000	R. 100	2176	20 years...	220	70	1070	240
5076	Still-born..	12	6	85	R. 10	2294	20 years...	220	120	1600	250
5085	2 years...	50	28	400	R. 20	3181	20 years...	200	100	1000	200
5087	3 months...	36	22	240	85	3294	20 years...	.....	100	1100	400
5106	Still-born..	11	5	140	6	3825	17 years...	300	160	1400	L. 175
5110	4 weeks...	13	9	80	8	3845	17 years...	150	40	1150	250
5112	1.5 years...	.....	.....	380	R. 28	3863	17 years...	210	50	1100	340
5134	Still-born..	10	1.5	25	R. 6	5024	19 years...	275	125	1600	R. 140
5138	Still-born..	17	17	170	R. 18	5077	19 years...	195	70	920	250
5142	27 days...	17	12	.....	14	3994	17 years...	215	.....	1660	395
5150	8 months...	32	16	220	.....	4246	20 years...	225	110	1400	320
5152	1 year...	47	26	325	R. 36	4410	19 years...	300	.....	1870	580
5155	Still-born..	28	7	95	27	5481	13 months...	40	.....	265	55
5156	16 months...	50	70	450	90	5522	14 months...	40	20	250	70
5157	Still-born..	27	9	80	35	5539	15 months...	54	14	250	72
5164	Still-born..	12	12.5	71	15	5244	16 months...	.....	15	270	80
5169	4 years...	100	35	820	R. 80	5540	23 months...	70	46	350	105
5187	4 years...	.....	26	480	R. 46	5448	1 year...	50	26	305	R. 30
5188	19 months...	41	37	320	R. 39	5367	1 year...	36.5	7.5	230	60
5176	Still-born..	18	3	22	4	5341	1 year...	33	22	200	R. 52
5177	2 weeks...	15	5.75	85	R. 13	5296	3 years...	.....	.....	330	R. 60
5186	20 days...	5.5	2.25	33	R. 6	5509	3 years...	95	30	560	R. 55
5195	13 months...	51	77	365	R. 30	5531	3 years...	60	15	.....	R. 70
5210	1 month...	26	6.85	174	24	5471	4 years...	65	.....	530	155
5214	New-born..	6.25	15.5	70	L. 4.5	5340	7 years...	87	32	500	R. 70
5218	.....	32	17	210	39	5387	7 years...	96	35	600	140
5223	18 years...	.....	95	1195	345	5480	9 years...	100	30	480	L. 65
5227	8 years...	105	75	740	180	5409	10 years...	140	40	1060	.....
3943	.....	50	30	320	90	5346	12 years...	120	80	940	R. 85
3963	15 years...	250	60	1120	260	5309	15 years...	.....	82	1490	R. 140
3968	1 month...	20	10	110	L. 12	5443	15 years...	310	110	1440	R. 125
3970	6.5 years...	120	35	580	95	5492	19 years...	370	110	1960	305
3795	11 months...	40	.....	.....	.....	5417	19 years...	260	250	1800	200
3415	Still-born..	40	20	210	40	5334	22 years...	270	100	1400	330

TABLE 4.—*Johns Hopkins Hospital, Colored Male Children.*

J. H. H. No.	Age.	Heart weight.	Spleen weight.	Liver weight.	Kidney weight.	J. H. H. No.	Age.	Heart weight.	Spleen weight.	Liver weight.	Kidney weight.
4103	21 months..	50	30	320	L. 50	4362	3 weeks...	25	40	175	20
4172	3 months..	25	20	200	30	4368	8 months..	20	50	120	40
4176	4 months..	20	10	120	30	4378	1 month...	.....	.....	150	50
4191	7 months..	25	15	200	60	4207	15 months..	22	60	300	.....
4198	6 weeks...	.....	.....	.....	70	4221	5 days...	20	8	60	30
2808	15 years...	210	65	1200	230	4241	14 years...	170	120	1260	215
2730	16 years...	300	170	1300	250	4252	8 days...	15	.....	100	20
2733	2 years...	50	50	350	40	4289	7 months..	.....	40	260	.....
2755	6 years...	50	20	450	150	4015	4 months..	25	15	220	60
2767	17 years...	250	.....	1530	350	4020	5 years...	80	50	480	L. 75
2777	10 years...	.....	100	950	300	4038	17 months..	.....	15	275	.....
2510	17 years...	350	.....	2170	420	4055	1 year...	50	40	350	80
2527	15 years...	.....	120	1530	.....	4058	21 months..	45	45	425	.....
2561	Still-born..	20	18	120	.....	4096	20 months..	45	20	240	75
2593	19 years...	240	.....	920	350	4097	1 year...	.....	30	.....	40
2445	17 years...	255	50	1520	330	4099	3 years...	55	40	400	R. 61
2466	11 years...	170	.....	1050	290	3999	6 months..	20	6	100	25
2351	15 years...	170	.....	1020	.....	3831	14 months..	50	40	.....	75
2123	8 months..	22	40	150	45	3844	2 weeks...	40	35	210	L. 50
2145	18 years...	350	.....	1820	426	4756	3 months..	35	20	285	L. 26
2164	3 years...	70	70	300	100	4760	5 months..	23	14	180	R. 20
2178	7 years...	140	65	850	R. 120	4762	27 days...	.....	11	180	33
2214	17 years...	.....	.....	.....	290	4764	10 months..	.....	40	220	R. 26
2085	11 years...	.....	45	680	200	4766	2 months..	20	8	115	R. 14
1997	19 years...	270	.....	1629	470	4768	2 months..	13	5	100	22.5
1838	20 years...	.....	150	1120	.....	4774	4 days...	19	14	95	31
1851	6 years...	70	20	350	100	4778	14 months..	38	24	320	R. 38
1700	Still-born..	.....	13	136	21	4785	Still-born..	20	8	150	22
1743	17 years...	180	.....	.....	.....	4601	Still-born..	11	4.5	45	14
1760	1 year...	40	30	250	50	4602	Still-born..	23	8.5	115	38.5
4401	22 months..	.....	28.5	275	.....	4607	3 days...	16	9	70	19
4402	5.5 months..	.....	20	190	50	4614	Still-born..	16	12	.....	R. 13
4408	6 months..	35.5	8	180	50	4632	Still-born..	11	18	85	24
4409	1 year...	33	21	375	75	4643	28 days...	18.5	10	185	53
4418	9 months...	.....	30	.....	.....	4649	Still-born..	15	8.5	50	L. 10
4455	16 years...	120	.....	700	200	4664	7 weeks...	23	10	150	.....
4458	2.5 months	16	9.5	105	27	4667	3 minutes..	4.5	0.75	.....	4
4461	Still-born..	.....	20	160	.....	4671	5 months..	27	23	200	R. 28
4468	2 weeks...	12	15	100	R. 9	4679	1 hour...	16	3	135	30
4471	6 months..	30	60	260	.....	4691	3 years...	92	40	520	116
4475	Still-born..	.....	60	14	.....	4693	2 days...	.....	2	220	L. 18
4481	5 months..	21	8	140	R. 17	4695	Still-born..	.....	9.5	125	36.5
4482	7 months..	21	7.5	140	R. 12	4510	Still-born..	.....	.....	180	36
4486	15 hours...	36	17	125	44	4512	11 hours...	12	15	165	22
4493	Still-born..	32	20	210	R. 16	4515	7 weeks...	35	20	150	27
4494	Still-born..	14	25	100	17	4520	Still-born..	6	2	35	7.5
3528	4 years...	50	25	300	100	4522	22 hours...	.....	6	60	14
3222	17 years...	190	.....	.....	.....	4528	1 hour...	.....	1	50	10
3282	14 years...	270	50	1330	260	4548	15 years...	300	210	1800	460
3120	5 years...	130	100	940	R. 90	4556	12 days...	14	2	55	15
3019	12 years...	.....	50	450	220	4560	7 months..	26	16	200	37
3045	17 years...	160	70	1200	.....	4900	6 years...	82	75	710	94
3051	10 months..	50	30	450	.....	4905	1 month...	.....	.....	.....	33
3071	10 years...	.....	60	790	.....	4907	Still-born..	6.5	2.8	45	16
3082	18 years...	.....	95	.....	350	4917	15 months..	20	12	.....	31
2900	17 years...	350	.....	2900	300	4928	5 weeks...	13	7	70	R. 11
2919	19 years...	.....	.....	1870	320	4930	30 minutes..	12	10	130	36
2924	17 years...	.....	110	1270	270	4940	Still-born..	16	7	55	18
2926	20 years...	300	200	1950	400	4947	22 months..	46	.....	300	R. 36
2975	15 years...	150	50	710	220	4955	12 hours...	29	9	32	35
4566	Still-born..	23	11	150	29	4967	8 months..	45	35	240	.....
4567	Still-born..	15	5	90	19	4976	Still-born..	21	16	160	L. 18
4593	Still-born..	18	3	60	16	4993	Still-born..	36	23	190	35
4594	8 hours...	22.5	13	110	18	4996	3 days...	30	17	145	42
4596	3 years...	.....	25	280	66	4802	Still-born..	.....	60	210	16
4332	3 years...	60	35	530	110	4810	Still-born..	25	14	225	44
4342	16 months..	.....	20	290	67	4814	7 months..	16	10	90	R. 18
4346	5.5 years...	.....	60	320	.....	4816	2 years...	55	8	380	64
4351	2 weeks...	10	3	35	20	4830	18 months..	45	26	.....	49
4355	17 years...	260	110	1380	320	4834	4.5 years...	90	25	440	87
4356	4 months..	47	22	260	47	4835	16 years...	200	.....	1350	280

TABLE 4.—*Johns Hopkins Hospital, Colored Male Children—Continued.*

J. H. H. No.	Age.	Heart weight.	Spleen weight.	Liver weight.	Kidney weight.	J. H. H. No.	Age.	Heart weight.	Spleen weight.	Liver weight.	Kidney weight.
4840	Still-born...	8	11	60	R. 5	3307	8 years...	150	50	700	210
4841	6 months...	43	20	210	88	3328	10 years...			1150	250
4850	6 months...	29	11.5	240	R. 23	3375	18 years...	150	90	890	
4852	23.5 months...		19			3395	2 years...	70	50	350	110
4871	4 years...	75	20	300	80	3345	21 years...	450		2070	470
4890	13 years...		91	823	210	3362	20 years...	390	290	1770	340
4700	Still-born...	28	15	200	40	3499	20 years...	350	70	1900	370
4702	7 years...	110	60	660	140	3633	21 years...	300	160	1820	440
4722	30 minutes...	10.5	3.5	47	15.5	3856	20 years...		25	1800	340
4744	5.5 months...	24.5	24	190	R. 24	4112	20 years...	200	200		340
4752	Still-born...	25	18	220	54	4277	21 years...	290	90	1500	380
4753	3 months...		50	300	100	4296	21 years...	250	250	2100	300
5011	Still-born...	18	6	125	R. 20	4889	20 years...	300	220	1380	350
5015	Still-born...	8	2	38	L. 4	4948	20 years...	340	110	1660	400
5018	Still-born...	3	2	40	8	4959	18 years...	190	175		400
5025	2 days...	18	9	110	25	5089	21 years...	380	350	2650	350
5027	Still-born...	25	40	200	11	1209	20 years...	325	170	2150	L. 160
5035	3 days...	10	4	70	8.5	1316	20 years...	250		2520	350
5039	4 months...	28	20	200	R. 21	1491	16 years...	230	110	1200	L. 100
5048	7 months...	33	14	190		1455	21 years...		130	1600	430
5050	6 months...	42	12	200		1600	21 years...	300		1530	350
5053	20 months...	60	50			1783	19 years...	200		1350	400
5075	1 day...	18	6	135	R. 12	1753	21 years...	300	190	1800	400
5079	6 years...	95	25	435	R. 42	1810	20 years...		200	1800	
5083	4 years...		41	600	R. 90	1844	19 years...	220			
5086	11 years...	160	120	1030	R. 120	2025	20 years...	320	170		
5100	Still-born...	13	8	45	16	2175	20 years...	350	100	1500	
5101	14 months...	50	28	260	R. 29	2184	20 years...	270		1950	370
5104	2 years...	65	20	430	R. 40	2254	21 years...	180	80	1350	
5107	17 months...	37	15	230		2424	21 years...		200	2450	450
5113	7 months...		26	280	R. 26	2687	21 years...	150		1100	
5116	Still-born...	8	6	90	R. 16.5	2604	21 years...	280			
5121	10 months...		46	450		2765	21 years...			2550	
5122	8 months...	50	25			2784	21 years...	220			425
5123	Still-born...		3	40		2793	21 years...	260		1600	
5128	3 years...	110	29	580	50	2716	20 years...	275	120	1600	
5136	18 months...	55	25			2877	20 years...	270	120	1300	290
5139	Still-born...	17	5			3153	20 years...	350	90	2020	340
5143	Still-born...	27	26			1770	Full term...	20	18	120	20
5153	Still-born...	26	9		32	1638	9 months...	40	15	230	50
5163	14 months...	75	28	350	88	1635	34 days...		10	120	35
5166	1 day...	24.5	7.7	110	20	1526	11 years...	100		920	250
5173	6 years...	90	30	445	R. 75	1580	New-born...	15	20	200	
5180	2.5 years...	64	45	340	100	1406	18 years...	270		2000	270
5171	Still-born...	11	4.75	62.75	21.75	1424	12 years...		60	970	260
5178	Still-born...	7	4.5	30.5	R. 6	1467	7 years...	110	100	500	
5192	Still-born...	20	7.5	65	R. 9.5	1497	13 years...	120	60	850	L. 70
5193	Still-born...	11	3.5	53	R. 6	5460	13 months...	49	35	340	60
5197	5 months...		11	180	R. 19	5238	13 months...	38	15	300	70
5198	2 years...	70	40	640	92.8	5502	14 months...	45	38	260	65
5204	Still-born...	7.2	13	45	16.5	5400	1 year...		24	380	R. 34
5215	5 months...	40	20	220	R. 21	5507	26 months...	50	25	460	75
5225	14 months...	39	27	360	34	5333	2 years...		25		
5233	11 months...	38	15	240	R. 21	5294	2 years...	43	22.5	350	
3938	11 months...	35	25	220	L. 25	5447	2 years...		50	450	150
3859	32 months...	55	20	300	L. 30	5327	3 years...	80	23		R. 80
3860	17 years...	55		600	125	5468	4 years...		80	480	R. 70
3898	7 months...	40	45	200	L. 50	5349	5 years...	110	30	500	R. 57
3788	7 years...	130	35	650	170	5245	7 years...	160	80	700	150
3405	5 days...	20	30	100		5392	17 years...	300	180	1780	
3416	4 months...		30	210	40	5270	21 years...	310	250	2350	440



TABLE 5.—Charity Hospital, White Female Children.

C. H. No.	Age.	Heart weight.	Spleen weight.	Liver weight.	Kidney weight.	C. H. No.	Age.	Heart weight.	Spleen weight.	Liver weight.	Kidney weight.
2108	19 years...	295	.....	1820	310	<b>1915</b>					
2911	17 years...	113	28	850	100	180	4 years...	90	70	500	130
2026	10 months...	28	14	.....	120	227	13 years...	170	170	1050	.....
<b>1913</b>						259	3 months...	20	10	150	30
107	14 years...	.....	190	1450	300	277	19 years...	330	.....	.....	.....
217	20 years...	340	190	1210	.....	301	3 days...	20	15	85	15
219	4 weeks...	25	20	140	B. 18	323	18 years...	.....	.....	1300	.....
<b>1914</b>						345	1 month...	20	10	90	15
62	2 years...	75	20	.....	B. 50	380	16 years...	100	150	800	.....
71	4 months...	14	11	65	.....	424	3 days...	20	10	95	25
85	3 years...	.....	35	440	B. 85	444	8 months...	30	20	200	55
105	2 years...	30	15	220	B. 55	<b>1916</b>					
123	.....	40	15	280	65	115	7 years...	.....	55	530	.....
137	3 years...	75	.....	400	100	164	3 months...	50	50	150	50
201	1 day...	25	10	140	.....	225	7 months...	50	35	230	100
206	2.5 months...	20	10	110	33	257	10 years...	150	.....	1030	170
272	2 years...	.....	.....	475	.....	277	3 years...	80	40	665	.....
300	9 years...	110	25	1230	.....	352	3 days...	35	35	150	65
328	5 days...	40	10	110	40	<b>1917</b>					
339	2 weeks...	50	30	160	.....	84	1 month...	50	30	.....	.....
350	2.5 years...	45	25	370	.....	238	1 day...	30	20	120	60
387	15 years...	190	100	1370	.....	<b>1918</b>					
<b>1915</b>						60	13 years...	110	70	800	170
3	19 years...	390	.....	1500	.....	108	13 years...	130	100	.....	210
72	9 days...	20	10	110	25	83	2.33 years...	55	50	480	90
74	3 years...	60	.....	470	.....	62	6 months...	40	20	145	44
77	7 days...	20	30	140	30	<b>1914</b>					
104	3 months...	20	15	160	.....	311	9 months...	.....	50	140	55
122	New-born...	15	5	60	17	<b>1918</b>					
135	13 years...	170	120	1020	220		10 years...	210	110	950	.....
149	20 years...	320	.....	.....	.....						

TABLE 6.—Charity Hospital, White Male Children.

C. H. No.	Age.	Heart weight.	Spleen weight.	Liver weight.	Kidney weight.	C. H. No.	Age.	Heart weight.	Spleen weight.	Liver weight.	Kidney weight.
2581	13 years...	170	.....	1580	230	<b>1915</b>					
2569	4 years...	21	15	425	56	38	7 months...	30	25	140	40
2447	19 years...	212	42	1500	.....	123	Newborn...	20	10	130	30
<b>1913</b>						167	10 days...	20	15	180	Horse-shoe.
114	7 years...	80	.....	490	220	241	2 months...	30	20	100	50
136	2 months...	24	4	75	29	252	8 months...	60	30	450	60
<b>1914</b>						276	11 years...	140	100	520	.....
7	5 years...	135	.....	.....	.....	281	6 months...	30	20	170	50
75	2 years...	60	25	410	60	291	6 weeks...	20	20	100	40
81	9 years...	125	115	920	200	295	2 months...	15	.....	150	.....
90	Died day born.	25	5	45	15	349	20 years...	230	.....	2400	340
154	7 years...	120	.....	580	180	369	20 years...	300	.....	2350	.....
168	2 months...	20	10	130	35	390	5 days...	25	20	150	30
185	Died day born.	.....	.....	.....	11	401	4 years...	70	30	400	110
198	9 months...	40	.....	250	60	404	15 years...	220	.....	1820	.....
217	6 months...	50	20	200	60	407	3 months...	50	.....	.....	.....
218	6 weeks...	20	.....	.....	.....	433	16 years...	200	.....	1570	.....
220	18 years...	.....	.....	1500	380	491	2.5 months...	40	15	225	20
227	21 years...	320	110	1600	.....	<b>1916</b>					
236	19 years...	225	100	1620	.....	13	18 years...	325	175	2100	.....
260	2 months...	20	10	150	45	32	5 weeks...	20	40	200	40
306	5 days...	25	10	115	25	73	3 months...	30	20	65	.....
321	6 years...	.....	.....	770	140	162	17 hours...	35	50	150	.....
324	5 months...	30	35	250	.....	209	New-born...	50	30	100	.....
332	18 years...	280	30	1780	418	213	7 days...	50	30	150	70
355	17 years...	195	.....	1350	295	280	.....	30	20	85	35
429	19 years...	160	90	1100	260	291	18 years...	390	.....	2040	.....
437	2.5 years...	50	110	750	116	316	10 years...	.....	.....	1305	.....
452	19 days...	20	10	100	30						

TABLE 6.—*Charity Hospital, White Male Children—Continued.*

C. H. No.	Age.	Heart weight.	Spleen weight.	Liver weight.	Kidney weight.	C. H. No.	Age.	Heart weight.	Spleen weight.	Liver weight.	Kidney weight.
<b>1916</b>						<b>1917</b>					
342	16 years...	190	.....	1800	.....	314	5 years...	60	35	410	50
344	New-born..	30	20	90	31	318	3 weeks...	30	25	200	50
347	22 months..	70	50	410	120	349	3 months..	85	50	375	78
379	2 months..	30	30	170	.....	<b>1918</b>					
<b>1917</b>						19	19 days....	40	40	170	54
37	Premature..	20	15	50	.....	52	4 months..	80	25	220	45
53	14 months..	65	40	290	80	54	20 years...	350	200	1400	.....
56	10 years...	180	65	1320	.....	68	10 months..	70	40	800	60
68	17 years...	370	190	1900	300	115	11+ years..	110	80	685	115
119	16 years...	230	200	1230	.....	2619	2+ years...	170	30	510	110
256	16 months..	80	40	460	150	2409	20 years...	311	120	1135	245
257	18 years...	260	.....	1500	.....	2588	1 year....	28	15	200	55
275	3 weeks...	30	10	80	40	2502	16 years...	254	283	2300	406
291	3 weeks...	25	18	110	45	2060	21 years...	311	170	1550	370
300	4 days....	30	30	60	25	2081	20 years...	270	140	1150	312

TABLE 7.—*Charity Hospital, Colored Female Children.*

C. H. No.	Age.	Heart weight.	Spleen weight.	Liver weight.	Kidney weight.	C. H. No.	Age.	Heart weight.	Spleen weight.	Liver weight.	Kidney weight.
2275	6 years...	170	85	.....	224	<b>1915</b>					
2408	18 years...	110	45	.....	96	97	20 years...	.....	130	1500	320
2637	19 years...	170	56	1020	224	113	21 years...	240	200	1570	.....
2232	21 years...	220	113	1020	.....	184	2 months..	40	10	110	.....
<b>1913</b>						197	4 months..	20	15	170	50
148	2 years...	50	35	325	.....	202	12 years...	100	50	740	150
168	19 years...	.....	.....	1700	320	209	3 years...	.....	50	370	110
177	3 weeks...	13	5	Very small.	19	235	19 years...	250	70	1040	250
182	3 days....	.....	4	122	30	238	2 months..	30	10	150	.....
192	19 years...	.....	90	1235	.....	247	15 days...	15	10	50	.....
207	4 months..	18	10	95	.....	256	New-born..	20	5	7	.....
<b>1914</b>						264	3 days....	15	5	110	20
17	4 months..	30	18	210	.....	278	1 year....	50	20	250	.....
55	7 years...	125	95	770	.....	282	18 years...	180	100	1250	.....
89	17 years...	235	75	1130	.....	341	18 years...	280	.....	1750	.....
95	3 months..	45	30	200	.....	342	5 days...	30	10	180	30
106	3 years...	70	40	600	150	363	18 years...	200	50	1200	.....
118	5 years...	90	40	.....	.....	378	New-born, premature	.....	60	.....	.....
131	2 months..	18	10	110	.....	392	5 months..	20	15	260	40
138	9 days....	20	7	135	.....	464	1.5 years...	20	20	270	60
160	20 years...	230	90	1530	.....	465	4 months..	25	15	180	.....
170	12 days...	20	10	80	15	468	1.5 years...	35	15	185	50
187	2 days....	30	15	135	62	498	4 months..	20	16	120	20
188	3 months..	20	9	130	.....	<b>1916</b>					
205	2.5 years...	55	40	380	80	8	19 years...	270	90	1725	.....
232	17 years...	215	100	1200	.....	9	17 years...	280	110	1700	.....
309	3 months..	10	5	55	42	42	1.5 years...	.....	25	400	105
313	4 months..	20	.....	125	45	49	4 months..	45	25	170	70
319	1 month...	35	23	135	.....	76	3 months..	59	.....	270	.....
322	19 years...	210	.....	1680	438	84	8 months..	60	90	230	100
366	19 years...	209	60	1810	.....	109	3 months..	50	.....	110	50
368	20 months..	17	10	.....	.....	111	12 years...	200	120	900	.....
396	2 months..	20	10	140	45	144	14 years...	230	140	1150	300
435	18 years...	.....	70	1220	.....	170	4 months..	50	50	300	100
<b>1915</b>						183	18 years...	400	120	1306	.....
13	4 months..	40	15	210	40	196	6 years...	225	100	800	.....
14	20 years...	210	180	1580	.....	197	18 years...	230	100	1450	300
16	2 months..	20	10	100	40	198	18 years...	.....	130	1850	.....
42	16 years...	160	90	970	190	199	1.5 years...	120	30	310	.....
61	6 months..	35	15	146	40	207	14 years...	150	.....	.....	300
84	5 months..	30	20	220	60	222	16 years...	350	150	1650	.....
						228	1 year....	50	30	220	90

TABLE 7.—*Charity Hospital, Colored Female Children—Continued.*

C. H. No.	Age.	Heart weight.	Spleen weight.	Liver weight.	Kidney weight.	C. H. No.	Age.	Heart weight.	Spleen weight.	Liver weight.	Kidney weight.
<b>1916</b>						<b>1917</b>					
230	16 years...	200	150	1350	350	185	11 days...	20	19	30	40
237	New-born...	50	80	220	60	191	4 years...	150			
242	6 months...	50	30	210		193	18 years...	300		1300	
245	3 weeks...	30	20	140	38	204	17 years...	250	100	1260	
252	5 months...	20	20	100	40	248	21 years...	380	180	2060	
266	17 years...	340		2100		252	18 years...	200	170	1400	270
333	21 years...	270	65	1075	330	292	23 months...	75	40	300	100
338	7 months...	45	30	180	75	326	10 months...	30	30	600	
355	18 years...	225		1430	360	337	15 years...	220		1120	
359	3 months...	40	30	150	60	339	16 years...	160	90	980	295
376	5 months...	70	45	190	85	359	4 months...	30	30	600	60
382	4 weeks...	25	16	50	40						
391	8 months...	50	30	220	40	<b>1918</b>					
<b>1917</b>						29	22 days...	22	14	70	28
3	2 months...	40	25	130	31	53	6 weeks...	45	10	200	60
19	1 year...	50	10	150	30	99	8 months...	40	40	285	40
114	10 years...	50	30	550		3153	5 months...	10	20	160	20
112	2 years...	50	30	280	100	3067	18 years...		100	2600	
123	17 years...	220	90		260	2016	12 years...	57			
172	8 months...	60	25	280		2383	21 years...	224			

TABLE 8.—*Charity Hospital, Colored Male Children.*

C. H. No.	Age.	Heart weight.	Spleen weight.	Liver weight.	Kidney weight.	C. H. No.	Age.	Heart weight.	Spleen weight.	Liver weight.	Kidney weight.
<b>1906</b>						<b>1914</b>					
2174	21 years...	170	170	1148	230	285	1 month...	17	23	190	47
2263	19 years...	295	200	430		289	20 years...	300	210	1650	
<b>1907</b>						292	4 years...	80	30	350	125
2354	17 years...	170		1800		304	New-born...	20	20	75	50
2418	16 years...	141	85	1350		323	5 months...	30	30	160	
<b>1908</b>						338	5 months...	30	30		70
2494	20 years...	311		674		348	17 years...	120		1270	
2642	20 years...	311	75	2090		349	11 months...	50	30		100
<b>1913</b>						357	11 years...	130	120	820	
127	17 years...		220	2675		358	15 years...	180		1600	
188	2 days...	45	20	190	30	388	7 months...	15	8	90	25
<b>1914</b>						405	21 years...	310	160	1940	
5	7 days...		5	80	30	421	3 months...	25	30	160	
61	2 years...	60		420		<b>1915</b>					
87	6 months...	20	20	160		6	6 months...	50	22	250	60
99	19 years...	260	210	1180		9	2 months...	20	10	100	20
102	14 months...	50	25	355	70	19	11 days...	20	20	120	30
119	16 years...	280			440	30	11 months...	50	60	370	90
139	5 years...	110	40	630	110	40	5 months...	25	20	150	65
149	3 months...	17	8	115	20	57	17 years...	250	200	1700	280
153	14 days...		8	80	40	91	5 years...	80	50	470	80
155	21 years...	285	177	1765		101	20 years...	230		1550	
165	16 years...	260		1400	330	108	20 years...		170	1450	
169	10 months...	45	20	150	55	110	18 years...	392	172	2382	
178	5 months...	30	20	155	42	163	17 years...	210	110	1100	360
195	6 months' gest....	14	3	75		165	20 years...	260	180	1570	
207	1 day...	25	10	100	22	174	21 years...	290	220	1650	340
216	17 years...		250	1250	240	221	9 months...	35	100	370	80
229	21 years...	340	145	1800		222	14 years...		140	1170	
241	6 years...	130	60	640	180	226	6 years...		50	650	150
248	20 years...	230	120	1550		233	4 months...	15	5	80	20
250	5 months...	20	10	180	60	243	3 months...	20	15	100	30
267	4 days...	25	5		22	244	4 months...	20	10	140	40
274	18 years...	190	80	1130		251	14 months...	50	10	200	80
277	New-born...	15	16	100	15.5	275	20 years...	320	70	1330	425
						285	4 months...	30	10	150	30
						214	New-born...	20	10	160	
						312	3 months...	20	10	100	20
						321	6 months...	30		200	

TABLE 8.—*Charity Hospital, Colored Male Children—Continued.*

C. H. No.	Age.	Heart weight.	Spleen weight.	Liver weight.	Kidney weight.	C. H. No.	Age.	Heart weight.	Spleen weight.	Liver weight.	Kidney weight.
<b>1915</b>						<b>1917</b>					
335	New-born...	20	15	150	30	72	4 years...	60	.....	810	.....
346	17 years...	250	.....	1820	.....	74	8 months...	80	60	350	.....
381	1.5 months	50	20	270	80	92	20 years...	450	250	1730	500
388	20 years...	320	120	1670	.....	105	1 month...	50	20	100	60
389	1 month...	.....	.....	70	.....	130	19 years...	340	210	2050	355
394	1.5 months	28	20	180	40	149	19 years...	290	.....	2000	280
417	7 years...	120	50	650	140	181	6.5 months	60	20	130	80
435	10 days...	20	15	70	.....	197	8 months...	60	.....	190	.....
437	1 year...	60	20	.....	.....	210	18 years...	250	.....	1140	280
478	19 years...	325	.....	1600	320	212	4 months...	30	30	.....	65
481	2 years...	44	15	320	52	222	1.5 years...	60	.....	640	.....
494	20 years...	230	.....	1600	.....	240	4 years...	300	.....	1680	.....
508	21 years...	300	180	1600	340	254	17 years...	240	.....	1200	.....
<b>1916</b>						263	2 months...	40	30	100	60
3	21 years...	265	.....	2015	355	271	12 years...	200	220	920	190
6	20 years...	285	145	1530	.....	279	7 years...	90	50	430	135
25	18 years...	255	80	1350	.....	281	6 months...	50	20	195	30
41	6 days...	20	30	300	40	302	5 months...	30	18	135	40
51	17 years...	180	105	.....	220	308	21 years...	360	110	1770	360
108	14 weeks...	40	60	240	.....	310	2 months...	.....	50	500	.....
116	12 days...	40	20	145	40	315	19 years...	315	260	2000	.....
118	3 months...	45	20	150	50	336	4 years...	100	60	600	140
127	7 months...	40	30	180	110	350	19 years...	340	270	1500	.....
133	7 years...	125	75	730	150	352	3 years...	80	40	440	210
136	20 years...	285	.....	1075	350	<b>1918</b>					
140	19 years...	.....	150	1450	.....	5	3 weeks...	40	20	135	40
171	16 years...	250	200	1550	345	10	16 years...	140	85	1080	.....
176	20 years...	300	.....	1700	.....	17	8 years...	150	40	480	160
188	4 days...	40	20	150	40	25	20 years...	.....	115	1500	.....
203	7 days...	.....	30	120	100	30	21 years...	250	.....	1750	.....
212	20 years...	250	.....	1475	300	39	20 years...	230	300	1550	.....
241	10 years...	110	220	1050	.....	44	2 days...	40	.....	110	60
247	21 years...	240	100	1230	.....	47	6 weeks...	45	115	240	70
311	4 months...	60	40	200	140	48	6 months...	25	.....	.....	40
314	3 months...	60	35	210	70	51	1 year...	70	20	.....	100
315	18 years...	270	130	1280	250	63	21 years...	380	130	1670	300
318	9 weeks...	20	20	75	.....	100	3 months...	35	20	100	30
330	19 years...	250	.....	1790	.....	122	3 months...	25	15	140	75
336	18 years...	290	125	1500	270	104	19 years...	500	.....	1570	330
346	New-born...	30	15	90	25	88	7 months...	40	30	205	R. 40
349	2 months...	30	20	115	115	85	17 years...	.....	.....	1360	360
360	17 years...	.....	140	1380	.....	50	15 years...	300	150	1250	400
364	3 days...	20	15	85	30	<b>1917</b>					
381	21 days...	45	25	140	45	299	9 days...	30	20	105	40
385	20 years...	.....	90	2330	.....	<b>1910</b>					
<b>1917</b>						3138	5 years...	.....	40	440	150
45	11 years...	210	120	930	.....						
58	21 years...	480	150	1700	.....						
65	7 weeks...	50	.....	250	50						

## CHARTS.

The 8 charts which follow were constructed from the data contained in the tables. In these the data from Baltimore and New Orleans are combined. *At birth* includes still-born, premature, full term and new-born specimens; 1-18 refers to the age in months at the time of death; 2-20 refers to the age in years at the time of death. In chart 5 four curves are shown after the age of 5 years, as explained in the text.

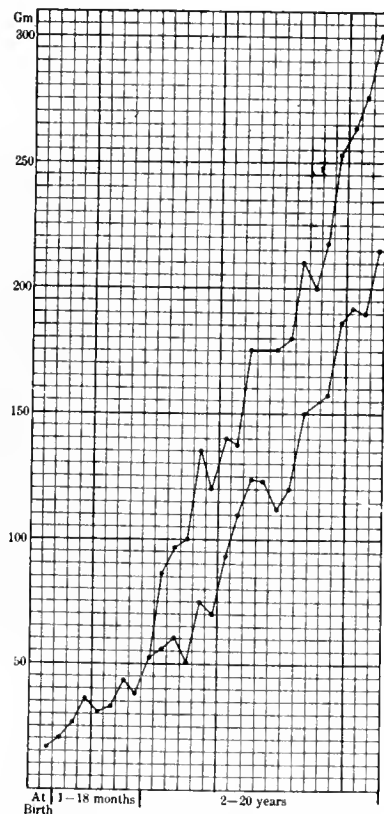


CHART 1. Heart weight and age, white and negro females.

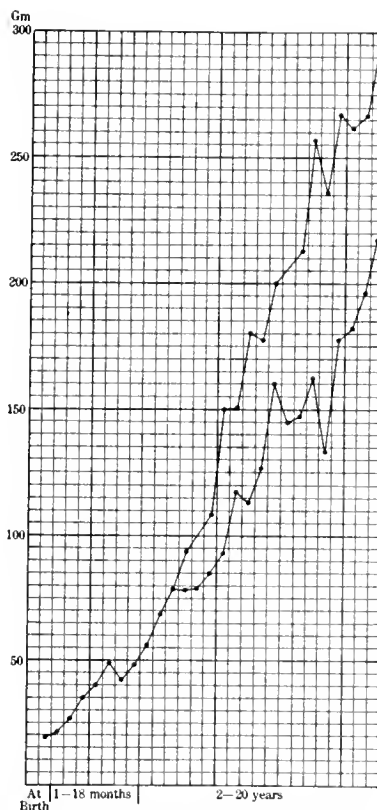


CHART 2. Heart weight and age, white and negro males.

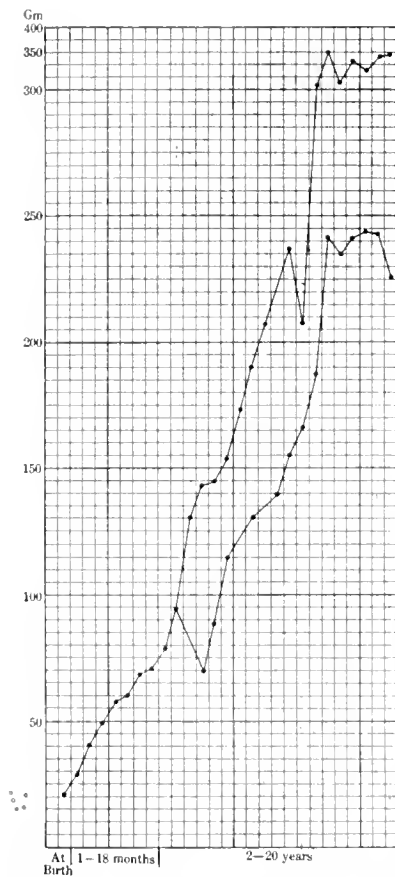


CHART 3. Kidney weight and age, white and negro females.

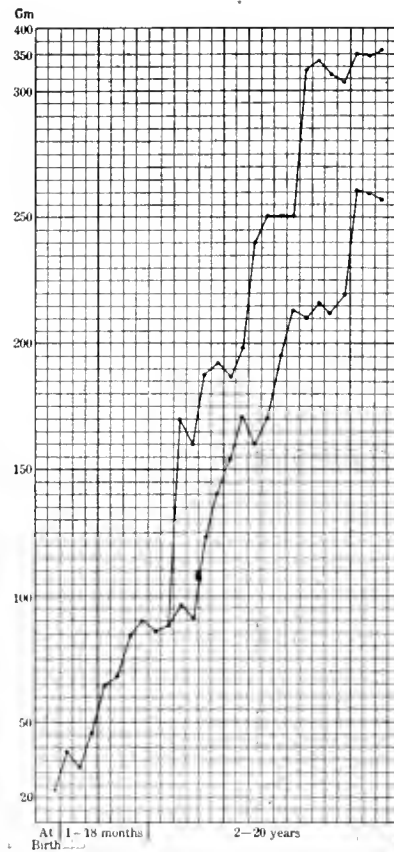


CHART 4. Kidney weight and age, white and negro males.

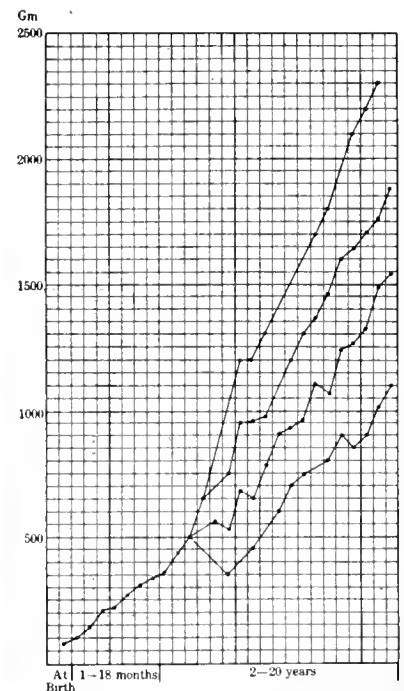


CHART 5. Liver weight and age, white and negro females.

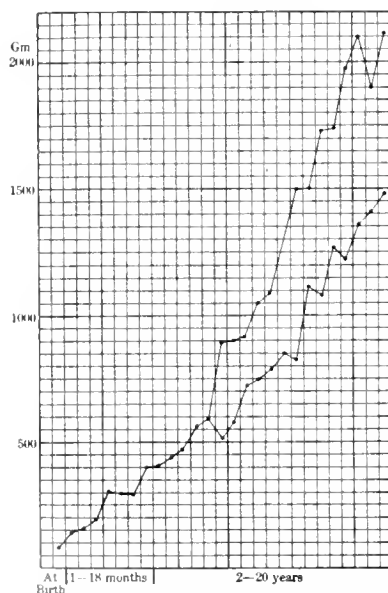


CHART 6. Liver weight and age, white and negro males.

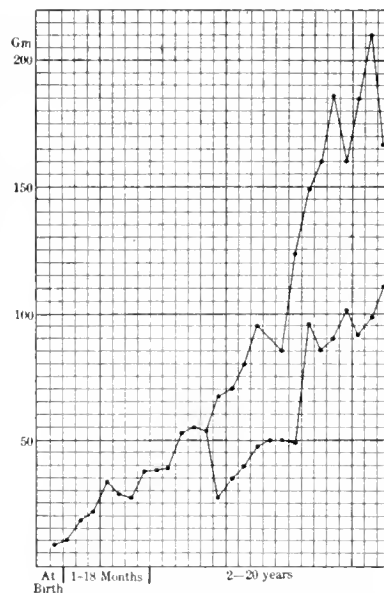


CHART 7. Spleen weight and age, white and negro females.

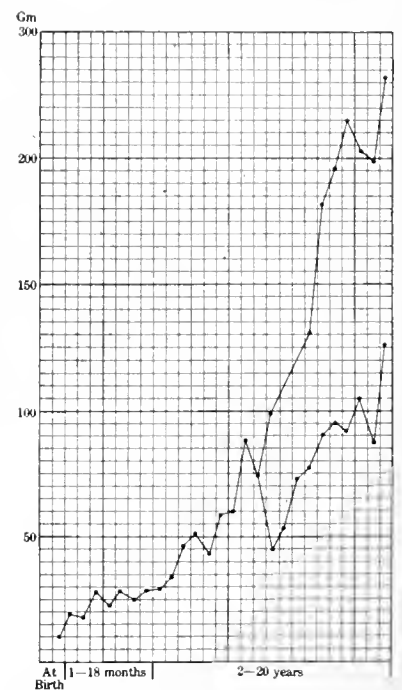


CHART 8. Spleen weight and age, white and negro males.

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CONTRIBUTIONS TO EMBRYOLOGY, No. 38.

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A MORPHOLOGICAL STUDY OF THE TRACHEAL AND  
BRONCHIAL CARTILAGES.

BY WILLIAM SNOW MILLER,  
*Professor of Anatomy, University of Wisconsin.*

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With two plates and eleven text-figures.





# A MORPHOLOGICAL STUDY OF THE TRACHEAL AND BRONCHIAL CARTILAGES.

BY WILLIAM SNOW MILLER.

Horner, writing in 1839, said that "at the orifice of each branch of the bronchia there is a semi-lunar cartilage, forming rather more than one-half of its circumference and having its concave edge upwards. The whole arrangement resembles somewhat the pasteboard of an eared bonnet, and is evidently to keep the orifice open." Passing over his reference to the feminine fashions of his day, we find in the above quotation the earliest description of the form of the cartilages found at the place where bronchi divide; moreover, a reason is assigned for the particular form of the cartilage.

In 1840 Jonas King published a short article "On the forms of the cartilages which keep open the principal divisions of the bronchial tubes." In a footnote he frankly gives the priority of description to Professor Horner and intimates that his paper was inspired by a demonstration Professor Horner made in Philadelphia to Mr. T. Wilkinson King "some months previous to the last edition of his work." Mr. King must have, on his return to London, imparted the information thus acquired to his namesake, who at once made it the subject of a special study. King found, however, that not all the cartilages present at the place where a bronchus divides had the characteristic saddle shape described by Horner but, "that a considerable number of varieties will be met with." His paper is illustrated with several small and unsatisfactory drawings of the cartilages.

An extended search of the literature fails to bring to light any other investigation of the form of the tracheal or bronchial cartilages; in no place has the author been able to find the cartilages illustrated in plastic form, and such illustrations as are found show the cartilages as irregular plates and are evidently schematic.

Henle's illustrations are poor, but his description is fairly good. He describes the cartilages as having the plates or strips sometimes with short prolongations, arranged, as a rule, transverse to the long axis of the bronchi; they may also have a longitudinal or oblique direction—

"Je tiefer hinab, um so mehr reduciren sie sich und um so weiter rücken sie aus einander, bis sie endlich nur noch als platte Ringe oder Halbringe um die Mündungen der Seitenzweige und als Stützen der die beiden Aeste einer gabeligen Theilung trennenden Scheidewand vorkommen."

The description of Waters is inexact and his illustrations still more so. He states that the cartilages—

"In the largest bronchial tubes are elongated transversely and placed more as they are in the bronchi, but in the secondary and subsequent divisions they are placed very irregularly, and are elongated longitudinally. . . . In the larger tubes, wherever a branch arises, a cartilage is always placed, and in the largest vessels the cartilage

forms two processes below, one of which belongs to each vessel of the division. The free margin of the cartilage, placed at the orifice of an air-tube, is always concave and sharp, and is surmounted by a band of yellow elastic tissue. At the points of origin of the smaller air-tubes, the cartilages exist as thin semilunar pieces, with sharp concave margin looking upwards; these becoming smaller, at length disappear."

Heller and v. Schrötter studied the cartilages which enter into the formation of the carina tracheæ and, although shown by flat drawings, they indicate better than any previous illustrations the bizarre forms which the tracheal cartilages often assume.

In a study of the carina tracheæ of the domestic cat, made by the author of this paper, a similar method of illustration was used, but while it served the purpose it did not in the end prove satisfactory.

Schäfer, describing the cartilages within the lungs, says:

[They] "no longer appear as imperfect rings running only upon the front and lateral surfaces of the air tubes, but are disposed over all sides of the tubes in the form of irregular-shaped plates and incomplete rings of various sizes. These are most developed at the points of division of the bronchia, where they form a sharp concave ridge projecting inwards into the tube."

Further quotations are unnecessary, for all the descriptions of the bronchial cartilages seem to have taken their coloring from the original descriptions given by Horner and by King. No one, however, has followed out the plastic representation of the cartilages first attempted by King.

In longitudinal sections of the trachea or of the bronchi a considerable number of so-called plates are seen (fig. 10) and it was a desire to see in plastic form the shape and arrangement of these plates that led to the present study.

Cuvier was the first to point out that the musculature of the trachea in some animals is inserted on the outer surface of the cartilages, in others on the inner surface of the cartilages. This apparently does not influence the shape of the cartilage but it led to the selection, for this study, of the tracheal and bronchial cartilages of man and of the guinea-pig (*Cavia cobaya*), both of which have the muscle attached on the inner surface of the cartilages. The study was begun on

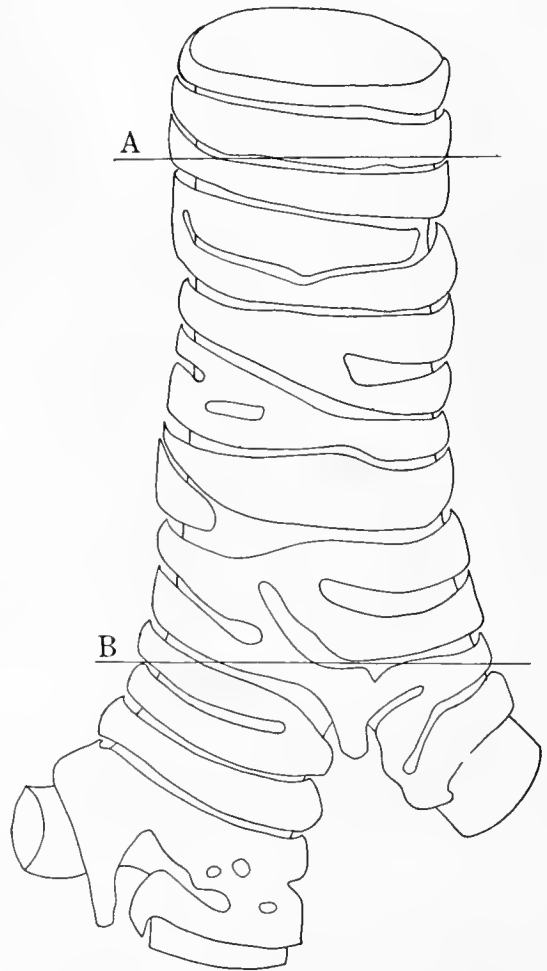


FIG. 1.—Outline of a reconstruction of the lower portion of the trachea of a week-old child. Only a part of the uppermost cartilage enters into the reconstruction. The lines drawn across the reconstruction show the plane of the sections indicated. Note the angle at the bifurcation, the greater diameter of right bronchus, and that the carina is at left of midline.  $\times 6$ .

the trachea and bronchi of a week-old child (fig. 1), but was later transferred to the guinea-pig, the latter having a smaller trachea and lung.

It must be understood from the first that animals of different species do not have the same form of cartilage in similar positions and that no two individuals of the same species have exactly the same form and arrangement of the cartilages. This point is well brought out in the studies of the cartilages of the carina tracheæ above mentioned. The last eight cartilages of the human trachea and the last five cartilages of the trachea of the guinea-pig were modeled. In each case it was found that only about one-half of the first of the cartilages entered into the model.

The outline of a transverse section of the trachea and of the cartilage in each of the models does not have the elongated horse-shoe shape of the figure given by Kölliker, or the flattened horse-shoe shape of the figure given by Sobotta; it is more nearly circular (figs. 2 and 5). As the carina is approached, the posterior membranous portion of the trachea flattens and eventually the section has an elongated oval outline; or it may even have, as is the case with the human trachea, a dumb-bell shape (fig. 3) before it divides into the right and left bronchus.

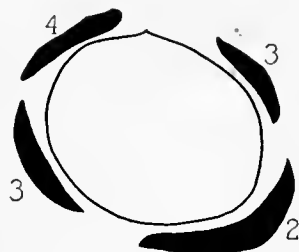


FIG. 2.—Outline of the section along the line A, in figure 1. Note circular outline of the section. The numbers 2, 3, 4 indicate the cartilages through which the plane of the section passes.  $\times 6$ .

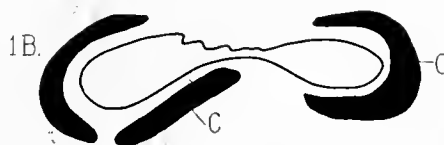


FIG. 3.—Outline of the section along the line B in figure 1. Note the outline of the lumen of the trachea; it has not only a dumb-bell shape, but there is also a marked anterior swing of what is to be the right bronchus. 1 B, section of the first right bronchial cartilage; C, C, sections of the carinal cartilage.  $\times 6$ .

#### HUMAN TRACHEA AND BRONCHI.

*Trachea.*—The cartilages of the human trachea present marked irregularities (fig. 1). The first of the cartilages is incomplete and must therefore be left out of the account; the two following cartilages are regular in their formation; the next five are either fused cartilages or are bifurcated, and have one or more irregular openings, evidently due to incomplete development, for they give passage to neither blood-vessels, nerves, nor gland ducts. The carinal cartilage is interesting, being formed by the fusion of tracheal and left bronchial cartilages (fig. 4); both elements present bifurcations and irregularities of outline.

*Right bronchial cartilages.*—The first bronchial cartilage on the right side consists of two elements which fuse as they arch around the mesial side of the bronchus. The next cartilage is a typical crescent. Immediately below this cartilage the eparterial bronchus is given off, and the cartilage placed at this point has a complicated structure, part of it belonging to the eparterial bronchus and part of it to the main stem bronchus. The portion belonging to the eparterial bronchus

has a prolongation which is associated with a small bronchus, arising from the eparterial bronchus, which is not shown in the outline. Three openings are present in the main portion of the cartilage which are probably due to incomplete development. From the description given by Horner and by King, a cartilage should be present in the angle formed by the eparterial bronchus and the main stem bronchus, having more or less of a saddle shape with the concave surface uppermost; but, as is the case in the guinea-pig, quite another type of cartilage is present.

*Left bronchial cartilages.*—On the left side the cartilages were not followed out completely, but, as far as they were followed, they formed a single fused cartilage made up of several elements, with numerous openings which, like those mentioned above, did not give passage to either blood-vessels, nerves, or gland ducts.



FIG. 4.—Reconstruction of the carinal cartilage shown in outline in figure 1. *A*, anterior view; *B*, posterior view. Note the extensive fusion of trachea and bronchial elements. This belongs to what is known as the "tracheo-bronchial left" type of carinal cartilage.  $\times 6$ .

The study by Heller and v. Schrötter, taken in connection with the study made by Miller, seems to indicate that the cartilages of the trachea and of the right and left bronchus in man are subject to a greater number of fusions and bizzare forms than is the case in the lower mammals; a more extended study of the lower forms might, however, prove this conclusion to be erroneous.

#### GUINEA-PIG.

The cartilages of the trachea and bronchi will be described first as they appear in a ventral view (fig. 12), and then as they appear in a dorsal view (fig. 13). The one shows what may be termed the bodies of the cartilages, the other, the ends of the cartilages.

*Trachea.*—In contradistinction to the human trachea, that of the guinea-pig presents no marked irregularities. The last tracheal cartilage has the triangular prolongation downward that is described as characteristic of this cartilage, but it does not enter into the carina tracheæ, the carinal cartilage being the first cartilage of the left bronchus.

*Right bronchial cartilages.*—The first bronchial cartilage on the right side forms a smaller segment of a circle than any of the other bronchial cartilages. It ends abruptly on the ventral side of the bronchus near the midline and has two small openings near its mesial end. The second right bronchial cartilage forms nearly a complete circle (fig. 14). The eparterial bronchus is given off just below this cartilage and the third right bronchial cartilage, belonging mainly to the right bronchus, will be considered in connection with its cartilages.

*Left bronchial cartilages.*—The cartilages of the left bronchus, seven in number, are quite regular in their formation and are placed slightly oblique to the long axis of the bronchus. The first cartilage, as already stated, enters the carina tracheæ

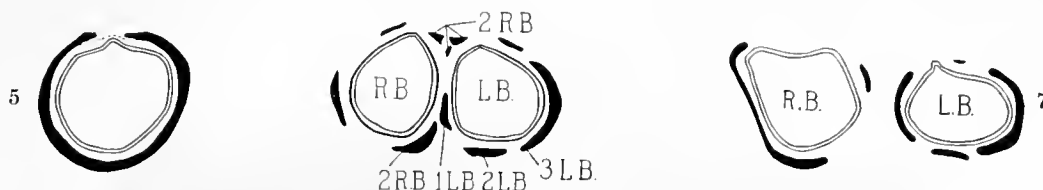


FIG. 5.—Section taken through the center of cartilage No. 3 in figure 12. This is a typical tracheal cartilage without any fission or fusion with an adjoining cartilage. The broken line extending from one end of the cartilage to the other indicates the positions of the muscle. Note the internal attachment of the muscle and that it is not inserted into the ends of the cartilage but some distance lateral to them.  $\times 6$ .

FIG. 6.—Section taken along the plane indicated in figure 12. The bifurcation of the trachea into the right and the left bronchus has taken place. *R. B.*, right bronchus. *L. B.*, left bronchus. 1 *L. B.*, lower portion of the mesial arm of the first left bronchial cartilage which forms the carinal cartilage. 2 *L. B.*, second left bronchial cartilage. 3 *L. B.*, third left bronchial cartilage. 2 *R. B.*, ventral and dorsal section of the second right bronchial cartilage.  $\times 6$ .

FIG. 7.—Outline of a section taken along the line indicated on figure 13, showing the relation of the cartilages to the right bronchus, *R. B.*, and to the left bronchus, *L. B.*  $\times 6$ .

and continues for some distance in that structure (fig. 6). The fifth, sixth, and seventh cartilages do not encircle the bronchus as extensively as do the second, third, and fourth cartilages.

Turning now to a dorsal view of the tracheal and bronchial cartilages (fig. 13), it will be seen that only the left tip of the first tracheal cartilage modeled is visible, and that the mesial end of the first bronchial cartilage on the left side is hidden from view by the expanded end of the second right bronchial cartilage. Of the remaining cartilages the left extremity of the last tracheal cartilage and the mesial extremity of the second right bronchial cartilage attract attention. The former of these is broadly expanded and has a well-marked posterior prolongation (fig. 13); the latter is expanded into an oval plate which fits into the angle between the right and left bronchus. Near the center of this expanded extremity is an oblong foramen (fig. 14) which gives passage to a small blood-vessel. It will also be noted that where the extremity of a cartilage is expanded the extremity of one of the adjoining cartilages is, as a rule, reduced in size—for example, the tracheal cartilages Nos. 2 and 4, and the bronchial cartilages Nos. 1 and 3 on the left side.

Considering the dorsal view of the trachea and bronchi as a whole, it will be noted that the interval between the ends of the cartilages in the trachea, which corresponds to the membranous portion, occupies the mid-line; as this is followed into either the right or the left bronchus it gradually rotates  $90^\circ$  and comes to occupy a mesial portion.

The angle which the bronchi form with the trachea differs from that which they form with the human trachea, in that it is practically the same for each bronchus; on the other hand, in man the right bronchus continues in nearly the same direction as the trachea, but the left bronchus has a more oblique direction. As a control for this point a number of celloidin corrosions were made and all showed the same mode of division. Narath's figure of a celloidin corrosion of the trachea and bronchi of *Cavia (Calogenys) paca* also shows the same characteristic. In still another point the bronchi differs from the human bronchi; in man the right bronchus exceeds the left in diameter, while in the guinea pig the two have practically the same diameter.

On the mesial surface of the left bronchus, just before the bronchus going to the left superior lobe is given off, there are present, in the interval between the ends of the main cartilages, two cartilaginous plates which are independent of any connection with the main cartilages. Such plates have been named by Luschka "intercalated cartilages." The more anterior of these two cartilages is long and narrow and is placed obliquely in the membranous intercartilaginous space; the other



FIG. 8.—Outline of a section taken along the line indicated on figure 12 showing the position of the various pieces of cartilage which enter into the formation of the cartilages shown in figures 12 and 13 along the eparterial bronchus. R. B., right bronchus. L. B., left bronchus. Ep. B., eparterial bronchus.  $\times 6$ .

FIG. 9.—Outline of a section taken along the line indicated in figure 13. The plane of the section takes in a portion of the bronchus going to the left lobus anterior and portions of the cartilage (A) shown in figure 18. L. B., left bronchus. L. l. a., bronchus going to left lobus anterior.  $\times 6$ .

cartilage, placed transverse to the long axis of the bronchus, is broad on the dorsal side of the bronchus, tapers as it arches around the mesial surface of the bronchus, and ends on its ventral surface just behind the seventh bronchial cartilage.

*Cartilages of the eparterial bronchus.*—The eparterial bronchus arises from the right bronchus opposite its third cartilage and quickly divides into five branches, which are distributed to the superior lobe of the right lung. The main stem of the eparterial bronchus, at the place where it leaves the right bronchus, is partially surrounded by two large cartilages. The one on the anterior (cephalic) surface consists of two elements, one of which belongs to the right bronchus, the other to the eparterial bronchus. In a ventral view of the bronchus (fig. 12) the bronchial element appears to be an independent cartilage, but when seen from the dorsal side (figs. 13 and 15) the fusion of the two elements becomes at once apparent.

The bronchial element differs from any of the preceding bronchial cartilages in that it encircles the stem bronchus spirally. It is irregular in outline and the lateral end tapers to a blunt point where it overlies the cleft mesial end. From the posterior surface of the lateral end there is a prolongation outward which fuses with a mesial prolongation from the dorsal surface of the element belonging to the eparterial bronchus.

This element is in the form of an elongated cap which incloses nearly three-fourths of the circumference of the bronchus and extends from the point where it leaves the right bronchus to the point where the apical branch arises. On the ventral side of the cartilage three deep notches and a single opening are seen; none of them gives passage to either blood-vessels, nerves, or glands. On the dorsal side is a deep notch which ends in a crescentic bay that gives passage to blood-vessels; the lateral margin is irregular, and the entire dorsal surface slopes toward the bronchial element with which it eventually fuses.

The second of the large cartilages associated with the eparterial bronchus belongs essentially to the main stem bronchus. In a ventral view (fig. 12) it appears as a band, placed transversely across the long axis of the stem bronchus, with a prolongation springing from its lateral margin which bears a slight resemblance to the bow of a ship and supports the eparterial bronchus in a manner somewhat similar to that by which the bowsprit of a ship is supported by the bow. In a dorsal view (figs. 13 and 16) this resemblance is not as pronounced as in the ventral view; this is due to a slight dorsal swing of the eparterial bronchus.

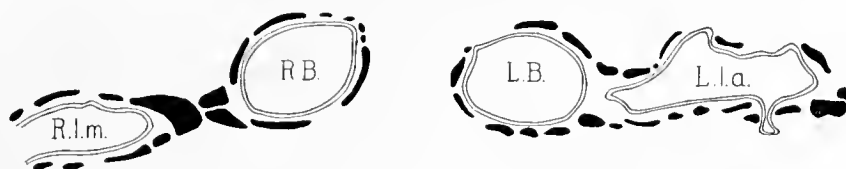


FIG. 10.—Outline of section taken along the line indicated in figure 12. *R. B.*, right bronchus. *L. B.*, left bronchus. *R. l. m.*, bronchus to right lobus medius. *L. l. a.*, bronchus to left lobus anterior. The relation of the various pieces to the complete cartilages can be made out without difficulty.  $\times 6$ .

The dorsal view also shows the close approximation of the two extremities of the cartilage, a foramen for the passage of a small branch of the bronchial artery and a small hook-line process arising from the posterior border of the lateral end of the cartilage.

The relation of these two cartilages to the eparterial bronchus is best shown in a view taken dorso-laterally (fig. 17). Here the capping of the eparterial bronchus by the first cartilage and the bracing of the bronchus by the second cartilage are clearly brought out. The relation of the second cartilage to the eparterial bronchus is also shown in the outline of a section taken through the anterior portion of the cartilage (fig. 7). This section also shows the position of the sections of the cartilages belonging to the left bronchus.

A longitudinal section (fig. 8) of the greater part of the eparterial bronchus before it breaks up into its principal branches shows the position of the various cartilages found along its course. The most interesting cartilage is the one that nearly surrounds the first of the small branches which arises from the dorsal side of the bronchus. It is shown in figure 8 in section, and in figure 13 it is shown reconstructed. This cartilage belongs primarily to the first dorsal branch; it also is the main cartilage associated with the second dorsal branch. The first branch takes a dorso-mesial direction, while the second branch takes a slightly dorso-

lateral direction. An elongated oval opening is seen in the portion of the cartilage situated between the first and second branches. Inadvertently the apical bronchus was not carried out far enough in the drawings of the reconstruction to show clearly that both the mesial and the lateral portions of this cartilage are also associated with the origin of this branch from the eparterial bronchus. This cartilage demonstrates a relationship between a cartilage and bronchi which has already been noted and which will frequently recur; namely, a cartilage may be associated with two or more bronchi.

Only a single cartilage is found between the eparterial bronchus and the bronchus going to the lobus medius that is exclusively a cartilage of the stem bronchus. This cartilage is deficient dorso-laterally, the interval between its two extremities being occupied by two small and one large, bizarre shaped, intercalated cartilages. Both extremities of this cartilage are broad; the dorsal extremity is perforated by a foramen for the passage of a branch of the bronchial artery.

The description of the cartilages has now reached the point where the bronchus going to the right lobus medius arises from the right stem bronchus, and the bronchus going to the left lobus superior arises from the left stem bronchus. These two bronchi arise nearly opposite each other from their respective stem bronchi. Figure 10 shows the outline of a section taken through the right and left stem bronchi, the greater part of the bronchus going to the right lobus medius, the bronchus going to the left lobus superior, and the position of the cartilages. In the outline drawing the cartilages appear as "plates;" that they are not plates but portions of well-formed cartilages is shown in figure 12 and 13, in which they are represented plastic.

It is not necessary to describe each cartilage in detail; a careful study of the ventral and dorsal views (figs. 12 and 13) will enable the reader to follow them individually without difficulty. It will be noticed that on both the upper (anterior) and lower (posterior) wall of each bronchus there are small cartilages which extend only a short distance around the circumference of the bronchus. These might, not inaptly, be called "plates," though the name introduced by Luschka, "intercalated cartilages," best describes them.

One cartilage belonging to the bronchus going to the left lobus superior deserves special consideration, for its form is unlike any other cartilage the author has studied. The first branch given off from the bronchus supplying the left lobus superior is the apical bronchus. This does not arise from the cephalic (upper) surface of the bronchus, but its origin is slightly rotated to the dorsal side of the bronchus. The cartilage in question is associated with this bronchus and can be easily followed in the ventral and dorsal views of the reconstruction (figs. 12 and 13), but is best seen in figure 19, which is a ventro-mesial view.

For convenience of description the cartilage may be said to begin near the posterior border of the bronchus as a slender bar which passes obliquely along the ventral surface of the bronchus. Arriving at the origin of the apical bronchus it divides into two broad arms which pass one on either side of the apical bronchus. The mesial arm arches over the main bronchus, then rotates so that its broadest surface is turned towards the main bronchus, and, passing along the corresponding



side of the apical bronchus, terminates in a pointed extremity. The lateral arm is the broader of the two, and after arching over the main bronchus and rotating in a manner similar to the mesial arm it passes along the lateral border of the apical bronchus to the posterior border of the main bronchus; it then widens out and, arching around the posterior surface of the main bronchus, continues along its ventral surface to terminate in a pointed extremity lateral to the bar of origin. The point of termination extends beyond the point of origin; the main bronchus is, therefore, surrounded by a cartilage which makes more than a complete spiral turn. This is the best example of a spiral bronchial cartilage which the author has found in any of the animals usually studied in the laboratory.

Spiral cartilages are frequently found in the Cetacea. Owen gives an illustration of a bronchial cartilage from the dugong, which makes four complete turns, but the cartilage is shown of uniform width and regular in its formation, while that above described is irregular in its formation and width and is associated with more than one bronchus.

On the mesial and dorsal side of the main stem bronchus, opposite to the origin of the bronchus going to the right lobus medius and the bronchus going to the left lobus superior, there are a number of intercalated cartilages which vary in size and shape. These are more numerous on the right side, especially in an area where there appears to be a deficiency in the development of the cartilages.

In the ventral view (fig. 12) the cartilages supporting these two bronchi are not well shown, but, as was the case with the eparterial bronchus, the dorsal view (fig. 13) shows clearly that in each instance the bronchus is supported by the same type of cartilage as the eparterial bronchus. That this is due to the wide angle at which these bronchi leave their main stem bronchi seems quite probable, for in no other instance do we meet with this type of cartilaginous support, the angle at which the remaining bronchi are given off being much more acute.

The supporting cartilage of the bronchus going to the lobus medius of the right lung is made up of two broad elements which have a very irregular outline and are perforated by two small openings, which do not give passage to either blood-vessels or nerves. The more anterior of these elements partially encircles the main stem bronchus, but the second element is confined to the dorso-lateral region of the stem bronchus. The supporting cartilage of the bronchus going to the lobus superior of the left lung consists of a single element. Immediately posterior to it, on the dorsal side, is a second cartilage of the same general shape, while on the anterior surface there is a long narrow cartilage which bears the same relation to the supporting cartilage as the second cartilage on the dorsal side. These two cartilages must be considered as forming a secondary series of supports for the bronchus going to the left lobus anterior. As this lobe is of considerable size and is attached to the main stem by a single bronchus, it undoubtedly requires a strong supporting apparatus, which is supplied by these three cartilages, and they occupy the same relationship to that bronchus as the two fused elements which support the bronchus going to the right lobus medius.

In the guinea-pig there are two cardiac (sometimes termed *infracardiac*) lobes, one for each lung; the right is the larger of the two. The bronchi passing to these lobes arise directly posterior to the cartilages just described. In the angle between the bronchus passing to either cardiac lobe and its main stem bronchus there is no distinct cartilage present; but capping each bronchus there is an irregular, horse-shoe-shaped cartilage, the lateral arm of which in each instance curves somewhat toward the mid-line and thus comes to occupy the position of a supporting cartilage.

We now find that the cartilages are no longer in the form of crescents arranged more or less parallel to one another; they are irregularly curved pieces scattered over every part of the circumference of the bronchi, with here and there small, variously shaped bits of cartilage interposed between the larger pieces. At the place where branches are given off, however, there are present cartilages of various types which I shall describe in detail, for it seems to me that they have an important relation to the mechanical support of the bronchi, the direction and freedom of their movements, and to certain densities which are often seen in roentgenograms of the lung.

In figure 20 a bronchus of the third order is seen arising from the ventral surface of the bronchus passing to the right cardiac lobe, just at the point where it has a slight curve towards the mid-line. The general direction of the bronchus is ventromesial. Three cartilages are grouped around this bronchus; first, a curved, triangular plate situated dorsal to the bronchus; second, a narrow, curved cartilage which arches around the anterior surface of the bronchus and is provided with a short spur which extends along the axis of the latter; the third cartilage is a somewhat wider plate which has a spur that arches around the posterior and lateral surface of the bronchus. From the arrangement of these three cartilages it appears that the bronchus possesses the greatest freedom of movement in a lateral direction.

In the case of the second ventral branch the opposite condition exists, for the bronchus is surrounded by a horseshoe-shaped cartilage, the arms of which pass dorsally on either side, permitting the greatest freedom of movement in a mesial direction. The more anterior of these arms extends in a curved direction along the dorsal side of the main bronchus and ends in a broad, flattened extremity which takes part in supporting a small dorsal branch of the main bronchus (fig. 13).

We now come to a typical saddle-shaped cartilage, as described by Horner, which is situated on the mesial side of a bronchus of the second order, which leaves the stem bronchus at nearly a right angle. The bronchus is directed posteriorly and the cartilage fits into the angle formed by the two bronchi. When viewed from the ventral side (fig. 13) the cartilage appears to be associated with only two bronchi, but when seen from the dorsal side (fig. 21) it is found to be associated with a third bronchus by means of a long mesial process which extends behind the small dorsal branch already mentioned.

During inspiration the bronchi elongate and the angle which they make with the main stem bronchus becomes wider; while in expiration the bronchi shorten and the angle becomes more acute. Under these circumstances cartilages of this type have a tendency to be drawn more snugly into the angle during inspiration

and in expiration the curved processes extending along each bronchus serve to support them in an efficient manner and to prevent their collapse.

In figure 22 we have two saddle-shaped cartilages placed, one on the ventral side, the other on the dorsal side of a small bronchus which is directed anteriorly. In figure 6 only the anterior cartilage can be seen, but in figure 22 the anterior and mesial side of each cartilage is shown. Each cartilage has two pairs of processes, the posterior of which fits over the main bronchus, while the anterior extends along the smaller bronchus. Between each anterior pair of processes there is a deep concavity which allows the bronchus to have a dorsal and a ventral movement but does not permit as much freedom of movement in a lateral direction. This type of cartilaginous support is intermediate between the simple saddle-shaped cartilage and the ring-like cartilage of the succeeding type.

The first dorsal branch of the main stem bronchus going to the right lobus inferior (fig. 13) is surrounded by an exceedingly interesting cartilage. Its shape is best seen in figure 23, which shows a portion of the main bronchus and a portion of the dorsal branch with the cartilage *in situ*. The cartilage is quite irregular in

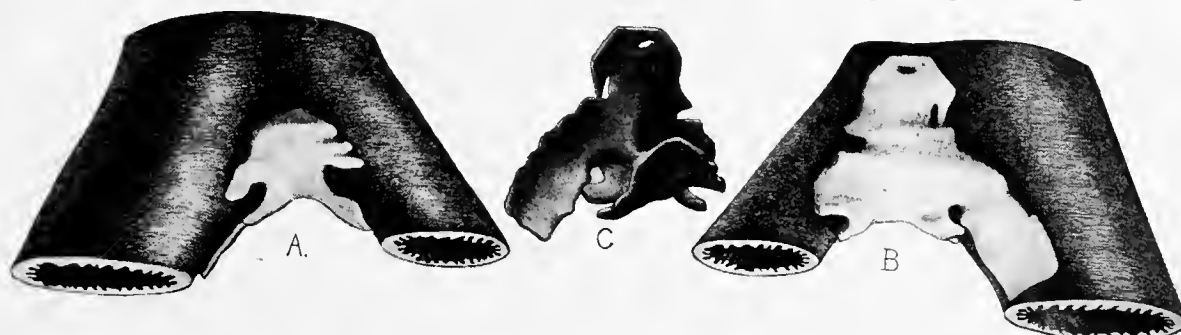


FIG. 11.—A typical saddle-shaped cartilage situated at the first division of the main stem bronchus in the left lobus inferior. A shows the cartilage *in situ* when seen from the dorsal side; B when seen from the ventral side; C shows the cartilage alone.  $\times 30$ .

shape and its two ends are placed, one slightly above the other, directly behind the dorsal branch. These ends do not fuse with each other, neither are they in direct contact. The opening through which the dorsal branch passes is ample and permits free movement of the bronchus in any direction. Another example of this type of cartilage can be seen surrounding the first dorsal branch of the left stem bronchus (fig. 13). With slight modifications I have found this type constantly associated with these bronchi.

In roentgenograms of the human lung a circular density is not infrequently noted where a branch is given off from one of the larger bronchi. This is quite often seen in what may be termed the middle zone (third) of the lung and is usually explained as being due to a bronchus which opens directly towards or away from the observer. That this is occasioned by the bronchus alone is not borne out by my own experience; for I have found it due to either a circular cartilage (figs. 23 and 24) or to two saddle-shaped cartilages (fig. 22). In either instance the bronchus must be viewed in the manner described above in order to produce the circular density. Bronchial cartilages, even in normal lungs, often show a slight calcification, and

when this is present it serves to intensify the density. A modification of the circular cartilage is seen in figure 24, where a spur from one of the ends of the cartilage is associated with a second and smaller bronchus. The cartilage itself is shown in figure 25.

In the lobus inferior of each lung the common type of cartilage in the angle between the main stem bronchus and its larger branches is the saddle-shaped cartilage (fig. 11); branches of the third and fourth order may have associated with them any one of the other types of cartilage.

When the smaller bronchioles are reached and the last trace of cartilage appears, it is not in the form of a thin plate, but as a short rod-shaped cartilage placed, as a rule, lengthwise along the bronchiole. This has been found to be the case, not only in the animals usually studied in the laboratory, but also in man.

In sections, a piece of cartilage is frequently found superimposed over one or more pieces of cartilage; this is due to the contraction of the trachea, or the bronchus, or to the plane of the section. Often pieces of different cartilages appear in a transverse section of the trachea or of a bronchus (fig. 2), giving the appearance of a series of plates arranged about its lumen; the preceding study, however, shows that they are integral parts of cartilage which have a definite form.

From the anatomical standpoint the act of respiration seems to be even more complex than the usual description. This morphological study of the cartilages shows that they constitute a mechanism for maintaining the normal position or capacity of the lung, and that a critical study of this mechanism (that is, the stress and strain to which it is subjected) is desirable. Associated with this is the relation of the bronchial musculature to the cartilages, especially at the point where bronchi divide. A study of the musculature at the carina tracheæ has convinced the author that it is by no means easily accomplished, though quite necessary for a complete understanding of the bronchial architecture.

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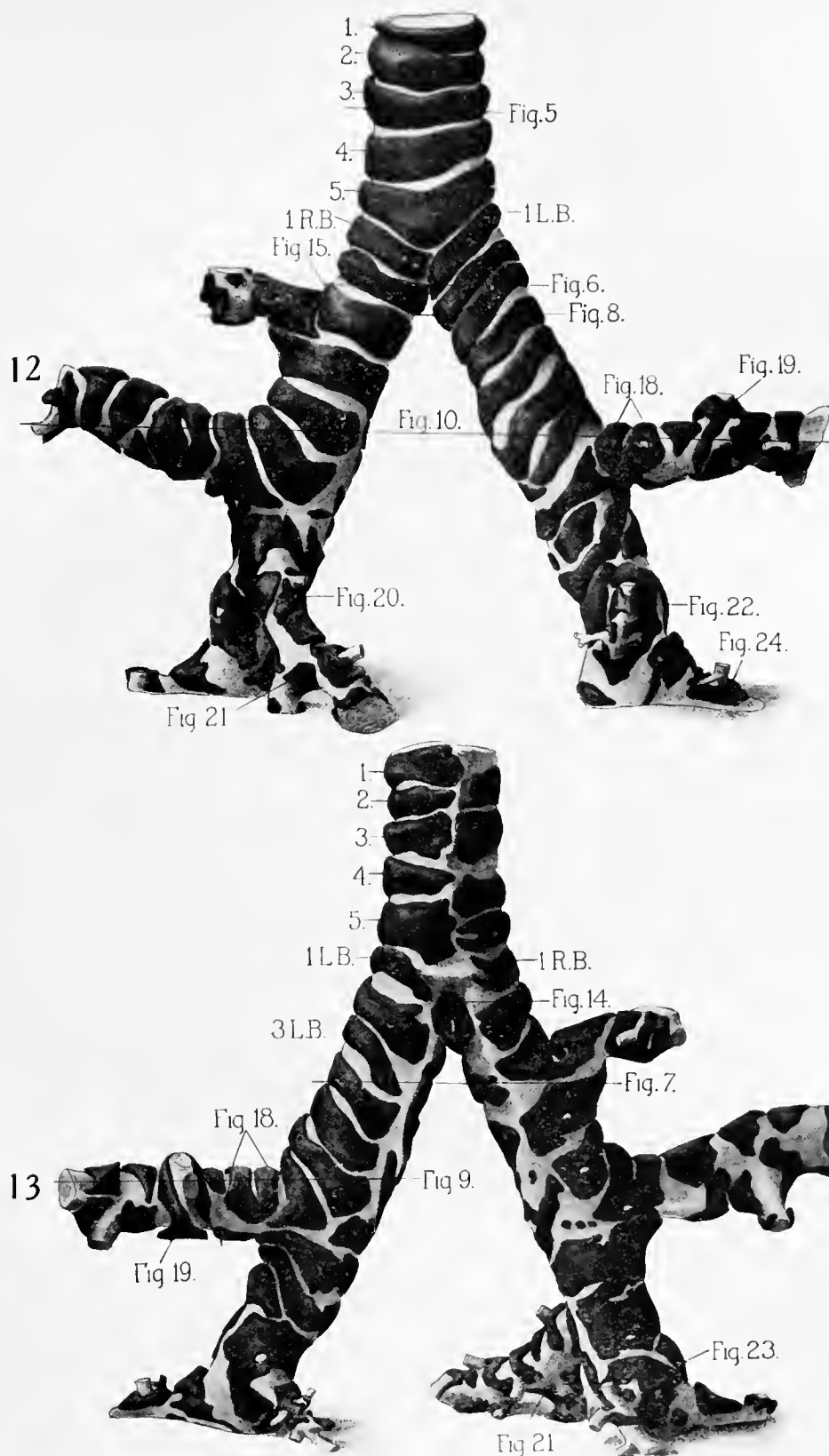


FIG. 12.—Ventral view of a reconstruction from serial sections of the last five cartilages of the trachea and of the right and left bronchi of the guinea-pig as far as the beginning of the first lateral branch of the main stem bronchus within each lobus inferior. Only a portion of the first cartilage entering into the reconstruction is shown. Note the angle at the bifurcation and the uniform size of the right and left bronchi. 1-5, tracheal cartilages. 1 R. B., first right bronchial cartilage. 1 L. B., first left bronchial cartilage. The lines drawn across the reconstruction show the plane along which the indicated figure is taken. The position of the cartilages shown in individual drawings is also indicated.  $\times 6$ .

FIG. 13.—Dorsal view of the preceding reconstruction. The plane along which the indicated figures are taken is shown; also the position of the cartilages which are shown in individual drawings.  $\times 6$ .



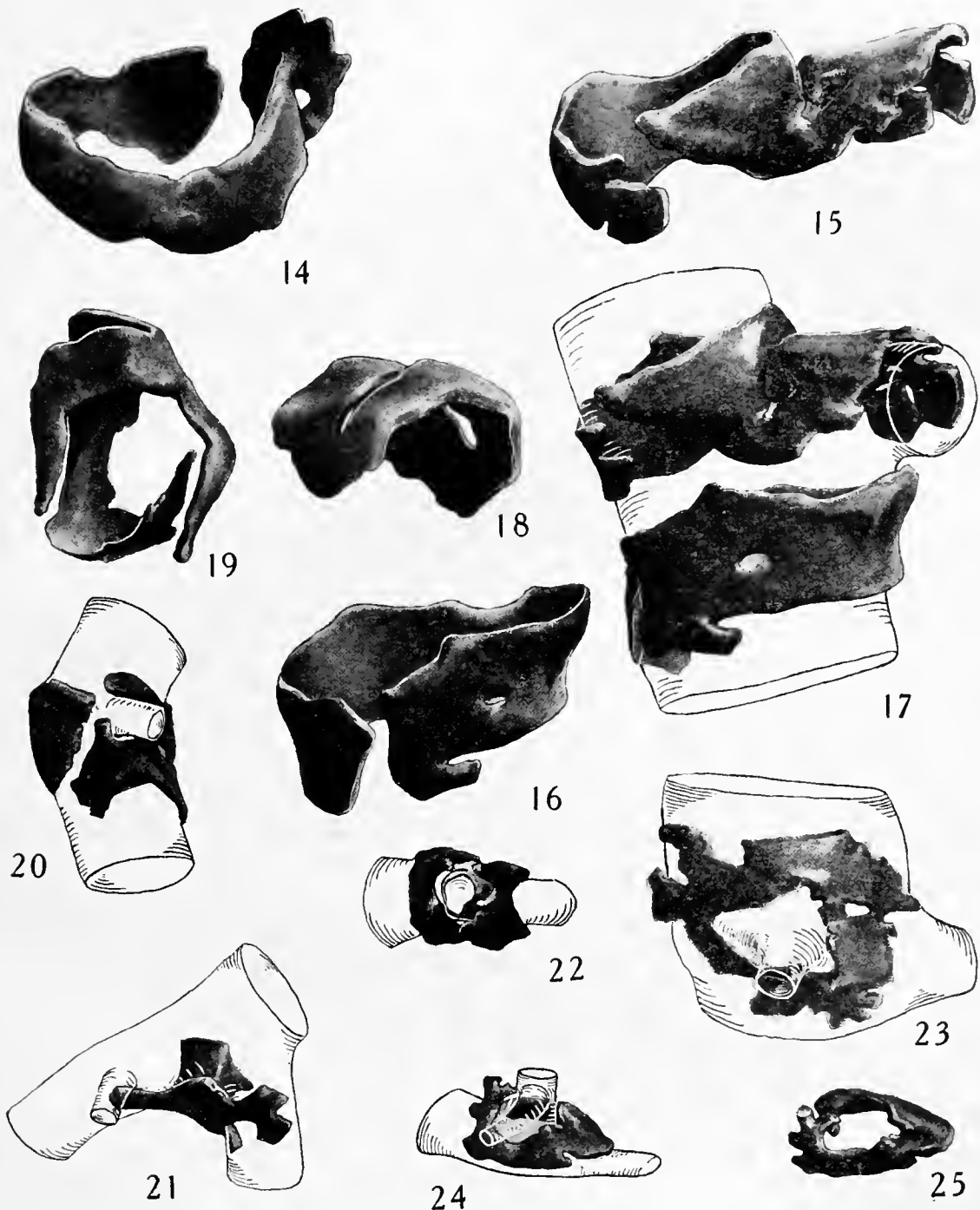


FIG. 14.—Ventro-mesial view of the second right bronchial cartilage. The expanded dorsal end of the mesial arm can be seen; also a portion of the oblong foramen mentioned in the text.  $\times 15$ .

FIG. 15.—Dorsal view of the fused elements that form the anterior cartilage which caps the eparterial bronchus.  $\times 15$ .

FIG. 16.—Dorsal view of the second cartilage associated with the origin of the eparterial bronchus.  $\times 15$ .

FIG. 17.—Dorso-lateral view of the two cartilages which are placed about the origin of the eparterial bronchus from the right bronchus.  $\times 15$ .

FIG. 18.—Dorso-mesial view of the first cartilage on the bronchus passing to the left lobus anterior.  $\times 15$ .

FIG. 19.—Ventro-mesial view of the cartilage described in detail in the text.  $\times 15$ .

FIG. 20.—Small bronchus surrounded at its point of origin by three special-shaped cartilages.  $\times 15$ .

FIG. 21.—Dorsal view of a small typical saddle-shaped cartilage shown *in situ*. Its relation to the two principal bronchi is clearly shown, also the prolongation by which it comes into relation with a third bronchus. The bronchi are shown as though transparent.  $\times 15$ .

FIG. 22.—Top view of a small bronchus surrounded by two saddle-shaped cartilages.  $\times 15$ .

FIG. 23.—A small bronchus surrounded by a ring-shaped cartilage which is quite irregular in its formation.  $\times 15$ .

FIG. 24.—A ring-shaped cartilage which surrounds a small bronchus and by means of a spur partially surrounds a second and smaller bronchus.  $\times 15$ .

FIG. 25.—The cartilage of figure 24 shown independent of its bronchi viewed from above and slightly from ventral side.  $\times 15$ .





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CONTRIBUTIONS TO EMBRYOLOGY, No. 39.

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THE CARTILAGINOUS SKULL OF A HUMAN EMBRYO TWENTY-ONE  
MILLIMETERS IN LENGTH.

BY WARREN H. LEWIS,  
*Professor of Physiological Anatomy in the Johns Hopkins University.*

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With five plates.

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## CONTENTS.

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	PAGE.
Introduction.....	301
The chondrocranium as a whole and its relation to other structures in the head.....	302
Dorsal aspect.....	304
Median sagittal aspect.....	310
Inferior aspect.....	312
Lateral aspect.....	313
The occipital cartilage.....	315
Occipital vertebra.....	317
The sphenoid cartilage.....	319
The temporal cartilage.....	320
Otic capsule.....	320
Mastoid cartilage.....	321
The ethmoid cartilage.....	322
Bibliography.....	323
Legends for figures.....	323
Abbreviations.....	324

# THE CARTILAGINOUS SKULL OF A HUMAN EMBRYO TWENTY-ONE MILLIMETERS IN LENGTH.

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## INTRODUCTION.

The human skull has for generations attracted the attention of anatomists. From the imaginative comparative anatomists, imbued with the doctrine of the segmentation theory, we have inherited a bulky literature, gradually passing into oblivion, on the segments of an unsegmented skull. Its effects, however, are still to be noticed in the attempts to imagine a segmentation in the unsegmented brain. In the slowly accumulating literature on the cartilaginous skull of man and other vertebrates another phase is manifested. It was anticipated that in the so-called primordial or cartilaginous skull there would be found many indications of the phylogenetic relationships, and by a comparison of the cartilaginous skulls of the various vertebrates Gaupp and his school expected to show this even more clearly. The theory expressed in the terse phrase, "ontogeny repeats phylogeny," formed the basis for such expectations. We have, however, gradually come to realize that there is more untruth than truth in it. For example, how is it possible to fit the conditions found in embryo No. 460 (Carnegie Collection), which are shown in figures 7 and 8, to the theory that "ontogeny repeats phylogeny?" Who would claim that our primitive ancestors had more brains than skull, except perhaps the few who believe in the downfall of man? And yet in this embryo the brain is enormous in size as compared with the cartilaginous skull or "primordial cranium." Even with the maximum development of the cartilaginous skull the conditions are essentially the same.

Comparative anatomy shows that in lower mammals and vertebrates the skull is relatively large as compared with the brain, while human ontogeny shows exactly the reverse. The whole assumption that "ontogeny repeats phylogeny" was based upon the erroneous notions concerning evolution that were prevalent before the present-day conceptions of the germ-plasm were introduced. If the various steps in evolution have come about primarily through the modification of the germ-plasm, then we should expect changes to appear in the egg and in the subsequent stages of ontogeny, and the entire development would thus be modified as much as the adult. There undoubtedly are fleeting indications of our primitive ancestors in the development of the embryo, but they are not very numerous and are usually extremely difficult of interpretation.

It is probable that in the phylogenetic history some sort of a membranous skull preceded the cartilaginous skull, and the latter preceded the osseous; but it is apparent from recent studies on vertebrate cartilaginous skulls that they no more form a phylogenetic series than do the adult skulls of the same species. The series

of cartilaginous human skulls with which we are now familiar, modeled from embryos varying in length from 13 to 80 mm., has failed to add much or any additional phylogenetic evidence regarding the form of the skulls of our remote ancestors. In fact, these cartilaginous skulls are as characteristically human as the adult skull is human. It is becoming more and more clear, as our knowledge of the anatomy of the human embryo increases, that both it and its various organs are at all stages as characteristically human as are the adult body and its organs. One can distinguish with ease between the cartilaginous skull of man and that of the ape, the pig, the cat, the rabbit, or the mole, as each is as characteristically formed as are the adult skulls of the same species. Homologies and similarities are to be found in the cartilaginous skulls just as in the adult skulls, but it is doubtful if much additional evidence of phylogenetic relationships will be revealed by a comparison of cartilaginous skulls.

The great need in the embryology of the human skull, as of other vertebrates, is a more complete and detailed series of the various stages, showing the gradual development from the primitive membranous stages to the adult. The present communication is concerned primarily with a particular stage that helps to fill in one of the many gaps still existing in the literature. The study of each stage is necessarily laborious, since it is practically impossible to picture such complicated forms without resort to models. The various structures in the head and neck, including the cartilaginous skull of this embryo, 21 mm. in length, were modeled with the plaster of paris technique.

Table 1 includes all of the human chondrocrania that have been reconstructed and described within recent years.

TABLE 1.

Length.	Probable age.	Author	Date.	Length.	Probable age.	Author.	Date.
<i>mm.</i>				<i>mm.</i>			
13	37-38 days...	Levi.....	1900	23	8.5 weeks....	Van Noorden.	1887
14	37-38 days...	Levi.....	1900	30	.....	Jacoby.....	1894
17	42-45 days...	Levi.....	1900	28	58-62 days...	Levi.....	1900
17	50 days.....	V. Noorden...	1887	30	.....	Faweett.....	1910
18.5	7.5 weeks....	V. Noorden...	1887	40	63 days.....	Macklin.....	1914
21	8 weeks.....	Lewis.....	1919	80	.....	Hertwig.....	.....

#### THE CHONDROCRANIUM AS A WHOLE.

The chondrocranium at this stage forms a continuous mass of cartilage and precartilage. It constitutes but a small part of the brain covering, as does that part of the adult cranium which is ossified in cartilage. One can best understand the cartilaginous skull by comparing it with that portion of the human adult skull which is ossified in cartilage rather than with the cartilaginous skulls of the lower vertebrates. To do this with accuracy and precision an extensive series of stages intervening between the stage to be compared and the adult should be at hand. Unfortunately, but few stages are known, although in man more has been done than with any other vertebrate. We have in human anatomy a large literature on the ossification centers of both the cartilaginous and the membranous bones. This is,

of course, a great help towards understanding the cartilaginous skull. Such a literature for any of the lower vertebrates is scarcely existent, and therefore very little is now to be gained through a comparison of the cartilaginous skulls of different species.

It is well to bear in mind the great difference in size between the cartilaginous skull of the embryo and that part of the adult skull ossified in cartilage. Table 2 gives a few comparative measurements. Unfortunately, the embryonic measurements are all from the one cartilaginous skull under consideration; it would be most desirable to have several such specimens, since the size of the skull varies considerably in embryos of corresponding ages. To realize the extent of this variability one has but to compare the length of the basal plate, from the foramen magnum to the hypophysial canal, in embryos of approximately the same age. Similar differences exist in the adult, and the adult measurements here used are the averages for six skulls.

TABLE 2.

Distance measured, in millimeters.	21 mm. embryo.	Adult.	Ratio.
Foramen magnum to ant. end nasal septum.....	4.2	93	1:22
Length basioccipital, pharyn. surface.....	2.0	26	1:13
Length basisphenoid, pharyn. surface.....	1.6	24	1:15
Length nasal septum, vomer edge.....	1.8	54	1:30
Foramen magnum to dor. sellæ.....	2.6	44	1:17
Dorsum sellæ to tip crista galli.....	2.6	45	1:17
Width basioccipital between otic capsules.....	1.0	25	1:25
Width basisphenoid; between lingulæ in the adult.....	1.2	30	1:25
Width basisphenoid; including alar proc. in embryo.....	2.1	30	1:14
Width between outer edges of optic for., cranial side.....	2.0	28	1:14
Width between outer edges of for. rotun. cranial side.....	3.3	44	1:14
Width between int. aud. meat., outer edges.....	3.0	54	1:18
Width between the tips jugular proc., lateral edge.....	3.6	84	1:23
Width between the lateral edge of the roots of the styloid.....	4.4	84	1:19
Width between tips of mastoid.....	4.0	110	1:28
Width between hypoglossal for., outer edge cranial side.....	2.0	29	1:14

These figures serve to indicate in a general way the greater immaturity of the anterior end of the cartilaginous skull at this stage. The adult basioccipital is about 13 times as long as the cartilaginous basioccipital; the adult nasal septum about 30 times as long as the embryonic nasal septum. In agreement with this we find that the basioccipital consists of more advanced cartilage than the nasal septum. In one of the following paragraphs I have stated reasons for believing that the alar process of the sphenoid at a later stage becomes incorporated into the body of the sphenoid; this is partly substantiated by comparing the ratios for the distances between the alar processes of the embryo and between the lingulæ of the adult with the width between the outer edges of the optic foraminæ or the distance between the foraminæ rotundæ (the latter formed in precartilage in the embryo) in both embryo and adult. These ratios are the same in each case, namely, 1:14, thus indicating that if the rate of growth in each of these regions of the sphenoid is the same the alar process must be looked upon as ultimately forming the lateral part of the body of the sphenoid. If, however, we compare the width of the basisphenoid in the embryo (not including the alar processes) with that of the

adult (including the lingulæ) it will be seen that the ratio is 1:25, which corresponds exactly to the ratio for the width of the basioccipital between the otic capsules in the embryo and in the adult. One would naturally expect the two to grow at about the same rate and, if this is true, then the alar process should not be looked upon as ultimately forming part of the basisphenoid. Factors such as the possible pressure of the otic capsules on this region of the basioccipital may, however, account for the discrepancy, since the ratio for the distance between the hypoglossal foramina (1:15) is about the same as that for the basisphenoid, including in the embryo the alar processes and in the adult the lingulæ.

#### DORSAL ASPECT.

The most familiar aspect of the chondrocranium is the upper surface of the base, shown in figures 1 and 2. These figures should be compared with a somewhat similar view of the upper surface of the base of the adult skull shown in figure 4, where the bone, ossified in cartilage, is colored blue. One is surprised, somewhat startled in fact, by the general similarity between the cartilaginous skull of the embryo and that part of the adult skull which is ossified in cartilage. The general resemblance is even more marked in later stages when the chondrocranium is more completely developed, as shown in the figures by Macklin and Hertwig of skulls from embryos 40 and 80 mm. in length. Again, if the dorsal aspect of the base of the entire skull, as shown in figure 3, is compared with that of the adult, new resemblances will appear. In figure 3 is shown not only the cartilage, but also the precartilage, blastema, and part of the dorsal membrane which enters into the formation of the brain capsule. In comparing the adult skull with figures 1 and 3, it must be borne in mind that these figures are drawn with the basioccipital horizontal and not inclined, as in the usual view of the adult skull or in the view of the cartilaginous skull shown in figure 2. At the caudal end of the cartilaginous skull is the basioccipital with the two lateral parts, or exoccipitals, one on each side. The exoccipitals are continued into the occipital squamæ or nuchal plates which are not yet united in the middorsal line, so that the foramen magnum is incomplete. The basioccipital is continuous in front into the basisphenoid, and from it the two wings project on each side. The mesethmoid is continuous from the basisphenoid, without line of demarcation, to the anterior end of the chondrocranium, and connected with it are the nasal capsules. Lateral to the anterior half of the basioccipital and fused with it are the large otic capsules corresponding to the petrous bones of the adult, each being separated from the exoccipital by the large jugular foramen and the mastoid plate.

The basioccipital consists of a flattened plate extending from the foramen magnum to the basisphenoid. The caudal edge bordering the foramen magnum is more deeply notched than in the adult. This notch is continued into a deep groove on the cranial side of the cartilage. At the apex of the groove is the opening where the notochord, after passing through the condensed mesenchyme which fills the groove, enters the body of the occipital.

Both in the embryo and in the adult each exoccipital arises from the side of the posterior half of the basioccipital and is perforated at its origin by the hypoglossal canal. In this embryo the right hypoglossal canal is divided on the cranial side into two parts by a cartilaginous bar; on the pharyngeal side there is but a single orifice, a condition not uncommon in the adult. The axis of the hypoglossal canal is nearly dorso-ventral in the chondrocranium, while in the adult it passes almost laterally.

The lateral borders of the foramen magnum are formed by the lateral parts of the occipital or exoccipitals and the squamæ. There is a general similarity in direction, in position, and in form of the exoccipital in the embryo and adult. Included in the lateral occipital and constituting much of it, as seen in this view, is the occipital neural arch, which is partially separated near its dorsal end from the exoccipital and squama by a fissure (the occipital fissure) which is filled with condensed mesenchyme.

That part of the exoccipital (the alar lamina) which continues upward from the jugular process is concave on its inner surface for the large transverse sinus. The sinus continues upward and outward, as in the adult, across the exoccipital and mastoid portion of the temporal, but turns forward over the dorsal margin of the otic capsule instead of backward over the squama of the occipital, as in the adult. At the bottom of the transverse sulcus the exoccipital joins the mastoid portion of the temporal. Along the upper part of this junction young cartilage is present, but towards the jugular foramen the two are joined by precartilage and at the jugular foramen by dense mesenchyme or blastema, which in older embryos changes into cartilage. There is no line of demarcation between the exoccipital and the squama. The latter continues upward from the exoccipital as a broad, curved plate of cartilage.

Above the region of the exoccipital the occipital squama is continuous with the mastoid portion of the temporal. There is no line of demarcation between the squama and mastoid, and therefore much confusion exists in the literature regarding this region. This has come about through the use of the term *parietal plate* for the plate of cartilage lying above the outer edge of the otic capsule and partly continuous with it. We naturally associate the word *parietal* with the membrane bone of the same name, and the assumption has been that the parietal plate disappeared at a later stage and was replaced by the membrane bone. Its fate, however, has never been carefully traced, and it is probable that this so-called *parietal plate*, which extends backward into the nuchal plate, is part of the mastoid, for in the adult the mastoid is continued upward, around the posterior and even the dorsal edge of the petrous bone.

The foramen magnum is incomplete dorsally except for a thin membrane of connective tissue, the dorsal membrane. In later stages (as shown by Levi, Hertwig, and Macklin) the foramen is completed by a bridge of cartilage formed by the junction of the occipital squamæ, which gradually extends around the brain. This cartilaginous bridge or band, usually called the *tectum posterius* or *tectum synoticum*, in older stages extends around the brain from the lateral occipital and mastoid plate of one side to the lateral occipital and mastoid plate

of the other, and corresponds to the nuchal plate of the adult occipital squama, which, as is well known, ossifies in cartilage. It seems quite unnecessary to introduce into human embryology a term applied to a somewhat similar bridge of cartilage, arching over the brain from one auditory capsule to the other, in the cartilaginous skulls of amphibia and reptiles. The nuchal plate in the human embryo is not homologous to the tectum synoticum of the reptiles, since in man it is primarily continuous with the lateral parts of the occipital, and in reptiles with the auditory capsules only. It would be better to avoid entirely the term *tectum posterius* in human embryology, and to use the term *nuchal plate*, or *squama*, as Levi has done, which at once gives to it its true significance in the higher vertebrates, in man at least.

The relations about the foramen magnum can be appreciated best by comparing figures 2 and 4. Figure 2 is drawn in the same position as the adult skull and the squama is intact. In its growth around the central nervous system it precedes that of the occipital neural arch and (as has been pointed out by Macklin) fuses with its fellow of the opposite side to form the primitive foramen before the occipital neural arches meet or nearly meet.

The large jugular foramen lies between the exoccipital and the otic capsule, as in the adult, extending laterally to the junction of the exoccipital with the mastoid cartilage, and medially to the junction of the exoccipital with the otic capsule. In the embryo and in the adult the same structures pass through the foramen, but both the nerves and the vein are proportionally much larger in the embryo. These structures have, however, approximately the same relations to each other; the transverse sinus occupies the posterior and lateral part, the vagus and the accessory nerves are just anterior and medial to it, and the glossopharyngeal is still more anterior and medial and lies in the notch on the posterior edge of the otic capsule.

The temporal cartilage, as in the adult, has an otic capsule or petrous part and a mastoid plate or mastoid part. The otic capsule forms one of the most conspicuous features of the cartilaginous skull, especially at this stage, for at this time the relatively small size of the occipital squama behind and of the orbital wing of the sphenoid and the ethmoid cartilage in front gives even greater prominence to the otic capsules than at a later stage when the squama and anterior end of the chondrocranium are fully developed in cartilage.

The otic capsule consists of a medial cochlear part fused with the lateral side of the anterior half of the basioccipital, and a lateral canalicular portion that forms part of the lateral wall of the chondrocranium. The otic capsule is broader in proportion to its length than the petrous bone. The internal acoustic meatus, the opening of the aqueductus vestibuli, and the fossa subarcuata are all relatively enormous at this stage. In the infant the fossa subarcuata also forms a relatively large depression.

The direction of the axis of the otic capsule or petrous portion of the temporal is very similar to that of the adult. The upper surface of the otic capsule, as already noted, contains the internal acoustic meatus, the opening of the aqueductus vestibuli, and the large fossa subarcuata under the superior semicircular



canal. In the adult these openings are on the posterior surface of the petrous bone. If the adult skull is rotated so that the cranial surface of the basioccipital is horizontal, as in figure 1, of the embryonic chondrocranium, it will be seen that the posterior surface of the petrous bone is directed upward and corresponds in position to the upper surface of the otic capsule as seen in figure 1. In the embryo one leaf of the tentorium cerebelli is attached to the anterior margin of the otic capsule, and in the adult the tentorium is likewise attached to the corresponding margin of the petrous bone, so that the upper surface of the otic capsule faces the posterior cranial fossa, as does the corresponding surface in the adult. All of the mastoid plate (except its most anterior tip) and the squama of the occipital are posterior to the tentorium and face the posterior cranial fossa, as do the mastoid and nuchal plates of the adult.

Surrounding the caudal and dorsal edges of the canalicular part, and continuous with it, is the mastoid plate or cartilage. This cartilage forms part of the outer wall of the chondrocranium and is interrupted by a large mastoid foramen. We have already noted the fusion of the mastoid plate with the exoccipital and occipital squama. It is also grooved for the transverse sinus, and the latter covers practically all of the inner surface of the cartilage. That portion of the mastoid cartilage above the outer edge of the otic capsule corresponds, therefore, more nearly to the upper part of the mastoid bone lying caudal to the upper part of the outer end of the petrous bone. This becomes the more apparent when we consider that the upper surface of the otic capsule, as seen in this aspect of the chondrocranium, presents both the internal acoustic meatus and the opening of the aqueductus vestibuli, and thus corresponds to the posterior surface of the petrous bone and faces the posterior cranial fossa.

The basisphenoid, which continues forward from the basioccipital without line of demarcation, occupies about the same relative position as in the adult. The dorsum sellæ, although well marked, is but imperfectly developed and consists mostly of precartilage and blastema, not indicated in figures 1 and 2 but shown in figure 3. To this blastema is attached the medial part of the tentorium. Between the dorsum sellæ and the tip of the otic capsule the abducens nerve passes forward towards the orbit. In front of the dorsum sellæ is the large shallow sella turcica. The hypophysial canal is seen in the center of the sella and contains remnants of the hypophysial stalk. Connected with the basisphenoid are the temporal and orbital wings. The greater size of the orbital wings in the cartilaginous skull is exactly what we would expect when it is taken into consideration that the part of the adult temporal wing which is ossified in cartilage is smaller than the adult orbital wing ossified in cartilage.

The temporal wings project laterally from the sides of the basisphenoid, just in front of dorsum sellæ and at a lower level than the floor of the sella turcica. These wings form a small part of the very incomplete floor of the lateral parts of what will later be the middle cranial fossa. Each lateral part of the middle cranial fossa, although indicated here by the depressed area between the orbital wings and the otic capsules, is entirely filled by the lower end of the embryonic tentorium

cerebelli. The boundaries of the middle cranial fossa are more clear in figure 3, where the lateral blastemal walls are shown. The temporal wing extends into this lateral blastema and thus completes the medial portion of the floor of the middle fossa. Between the temporal wing and the otic capsule is the large middle lacerated foramen, through which pass the internal carotid artery and the mandibular nerve. The facial nerve, with the geniculate ganglion and the greater superficial petrosal nerve, also passes through the foramen. The large otic ganglion lies across the foramen (figs. 12 and 13). The precartilaginous tissue attached to the anterior aspect of the temporal wing completes the foramen ovale and separates it from the supraorbital fissure. Between the temporal wing and the orbital wing, with its precartilagage, is the large supraorbital fissure, and through it the oculomotor, trochlear, abducens, and ophthalmic nerves pass into the orbital cavity (fig. 15).

The embryonic tentorium cerebelli consists of two lateral prismatic masses of mesenchyme connected across the midline by a band of condensed mesenchyme that extends upward from the whole breadth of the dorsum sellæ. The bases of the prisms are against the lateral walls of the membranous skull and the apical edges at the membranous band that extends upward from the dorsum sellæ. The lower end of each prism fills one of the lateral depressed areas of the potential middle cranial fossa, between the otic capsule in back, the orbital wing in front, and the lateral wall of the membranous skull. The posterior wall of each prismatic mass of the tentorium is composed of a thin layer of condensed mesenchyme, and its lower edge is attached along the anterior edge of the otic capsule and extends upward. The anterior wall of each prismatic mass, likewise composed of a thin layer of condensed mesenchyme, is attached to the posterior border of the orbital wing and its precartilagage and extends upward. These two membranes come together along the medial apical edge where they are continued into the medial connecting band. Laterally, the two membranes fuse with the lateral membranous wall of the skull. The interior of each prism is filled with loose mesenchyme in which are imbedded the semilunar ganglion, large blood-vessels, and nerves. The trochlear and oculomotor nerves pass from above downward through the entire length of the prism. The tentorium obliterates entirely the potential middle cranial fossa. Later, when this fossa develops, the anterior wall of the tentorium must be pushed down and back into the floor and against the posterior wall of the fossa.

Each ala temporalis consists of two distinct parts, a medial alar process joined to the basisphenoid by young cartilage, and a lateral part which is, more strictly speaking, the cartilaginous temporal wing. It is attached to the under surface of the alar process by condensed mesenchyme and lies at a lower level (figs. 1, 2, 3, 5, 6, 10, 11, and 14). Each alar process is usually regarded as forming the lingula, but since the internal carotid artery, which enters the cranial cavity between it and the apical end of the otic capsule, passes over its cranial surface, it seems probable that it gives rise as well to that part of the basisphenoid which forms the carotid sulcus. The relation of the greater superficial petrosal nerve to the alar process also indicates that the latter is incorporated into the body of the sphenoid

since, in the passage of the nerve from the geniculate ganglion to the sphenopalatine ganglion, part of its course is just beneath the alar process medial to the medial end of the temporal wing proper. It is here that the junction of the body with the temporal wing and the pterygoid process occurs and where later develops the pterygoid canal (fig. 13). Only the lateral part of the ala temporalis of the embryo, then, corresponds to that part of the temporal wing of the adult which ossifies in cartilage. In the adult the carotid sulcus and the lingula are at a higher level than the temporal wing, a condition which exists in the embryo if we consider the alar process as forming a part of the body of the sphenoid.

Since most of the temporal wing and both plates of the pterygoid process are ossified in membrane, the cartilaginous wing, even when fully developed, represents but a small part of the temporal or greater wing and pterygoid process of the adult. The maxillary nerve lies in front and the mandibular nerve behind this cartilage. In later stages the cartilage grows around the maxillary nerve and separates it from the supraorbital fissure, thus forming the foramen rotundum (figs. 3 and 10). Cartilage does not grow around the mandibular nerve, and the foramen ovale is said to be formed by membrane bone. At this stage, therefore, the large cleft between the temporal wing and the orbital wing represents more than the supraorbital fissure.

The alar process and the temporal wing proper each has its own center of chondrification, as pointed out by Bardeen and Fawcett. The alar process chondrification unites with the basisphenoid before the temporal center unites with the alar process.

The cartilaginous orbital wing, like the temporal wing, is very incompletely developed, as will be seen by comparing it with the older stages of Levi, Maeklin, and Hertwig. It consists of two parts, a proximal or basal part, and a lateral, sickle-shaped part, which springs upward, outward, and forward around the optic nerve and has much the same general position as that part of the orbital wing immediately about the optic nerve of the adult. The basal part of the orbital wing is connected to the basisphenoid by young cartilage and to the lateral part by cartilage not quite so far advanced as that in either the basal part or the lateral part. It seems probable that both parts of the orbital wing may arise from independent centers of chondrification. From the general position of this basal part and its relation to the optic nerve and to the general mesenchyme of this region, and from the fact that it gives origin to all of the muscles of the orbit except the superior oblique, I think it must ultimately become incorporated into the body of the sphenoid. The sickle-shaped lateral part of the orbital wing is intimately related to a larger precartilaginous part indicated by the green-colored structure in figure 3. This precartilaginous part shades off into the frontal blastema and there is in reality no sharp line between the two. The cartilaginous and precartilaginous parts together have somewhat the same form as the orbital wing of the 40 mm. embryo described by Maeklin.

In the cartilaginous skull the optic foramen is incomplete but, as shown in figure 3, is completed by the precartilage extending from the tip of the orbital wing to the anterior part of the body of the sphenoid.

In figure 1 it can be seen how the anterior end of the basisphenoid rapidly narrows and continues into the mesethmoid. Both in figures 1 and 2 the dorsal thin edge of the mesethmoid can be seen; it corresponds in the adult to that part of the mesethmoid which bisects the posterior part of the cribriform plate. The mesethmoid extends into the large crista galli, which consists mostly of precartilage. In figure 3 the position of the future cribriform plate is indicated by the large olfactory foramina, one on either side of the short dorsal edge of the mesethmoid. On either side of the mesethmoid, and connected with the anterior edge in front of the crista galli, are the lateral wings of the nasal capsules. As seen in figures 1 and 2, the posterior edge of each capsule is presented.

The anterior cranial fossa is not evident in the cartilaginous skull. In the combined cartilaginous and membranous skull shown in figure 3 its limits are clearly indicated. The floor of the fossa is formed by the precartilage of the orbital wing, frontal blastema, and dorsal membrane, and in the center are the mesethmoid and crista galli and the olfactory and ethmoid foramina. Figure 3 shows not only the floor but also a considerable extent of the anterior wall formed by the dorsal membrane.

#### MEDIAN SAGITTAL ASPECT.

In order to compare the view from the median sagittal plane shown in figure 5 with a similar view of the adult skull, the latter should be rotated so that the cranial surface of the basioccipital is horizontal. In the median sagittal section of the adult skull before me the pharyngeal edge of the basioccipital measures 26 mm., the basisphenoid 24 mm., and the upper edge of the vomer (from the basisphenoid to the anterior nasal spine of the maxilla) 54 mm. The latter distance corresponds to the ventral edge of the mesethmoid. In the cartilaginous skull the corresponding distances are 2 mm., 1.6 mm., and 1.8 mm. The ratios, then, are respectively 1:13, 1:15 and 1:30. The basisphenoid is thus proportionally shorter in the embryo at this stage than the basioccipital, and the mesethmoid much more so. This is what one would expect to find in the less differentiated regions at the anterior end of the skull.

The angles which the pharyngeal surface of the basioccipital make with the same surface of the basisphenoid, and the latter with the mesethmoid, are practically identical with those found in the adult. Since the edge of the basisphenoid is concave, a straight line connecting the two ends was used. It is interesting to note in this connection that the angle made by the cranial surface of the basioccipital, projected sagittally on to a line parallel with the upper edge of the zygomatic arch, is almost exactly the same in this embryo as in the adult. Such measurements serve to indicate that the cartilaginous skull is laid down from the very beginning on much the same plan as that part of the adult skull ossified in cartilage.

The basioccipital shows in its median sagittal section two parts—a thin caudal part lying beneath the notochord connecting the posterior part of the two lateral thickened masses of the body from which the exoccipitals arise, and a long wedge-shaped part in front of and above the notochord; this is thickest where it joins the basisphenoid. The diagonal course of the notochord through the basioccipital and its reëtrance into the basal plate at or near the junction of the basioccipital with the basisphenoid is also clearly shown. The three roots of the occipital neural arch arise from the posterior half of the basioccipital and unite lateral to the hypoglossal foramina into a tapering, rod-like cartilage. The serial relationship of the occipital neural arch with the cervical neural arches is very apparent in figure 5. The dorsal tip is in line with the dorsal tips of the cervical neural arches. The occipital squama is very prominent in this view; it appears to continue upward from both the exoccipital and the otic capsule, and even to project in front of the otic capsule. There is no line of demarcation between it and the mastoid plate which encircles the posterior and upper edges of the canalicular part of the otic capsule and thus no definite limits can be given to either cartilaginous area. The anterior part of this plate is usually called the *parietal plate*, a term which we have already discussed and discarded, since it is not apparent that it includes anything more than what might be embodied under the term *mastoid plate*. In considering this region it should be borne in mind that figure 5 is drawn with the basioccipital horizontal. The large mastoid foramen (capsulo-parietal fissure) lies at the bottom of the transverse sulcus and interrupts the continuity of the mastoid plate. Above the region of the otic capsule the masto-squamal plate is grooved for the endolymphatic sac. Whether this groove lies on the mastoid or squamal part is uncertain. The fate of the various regions of the masto-squamal plate will remain obscure until we have a more complete series of stages.

The surface of the otic capsule presented in this view corresponds to the posterior surface of the petrous bone and faces the posterior cranial fossa. Its diagonal position also corresponds to the adult when the latter is seen from the same angle. The cochlear part is mostly above the level of the basioccipital, projecting but slightly below the level of the latter. The canalicular part shows the position of part of the superior semicircular canal in front and of the posterior canal in back. The endolymphatic sulcus serves to mark the position of the common duct.

The medial section of the basisphenoid extends only to the hypophysial canal and a diagonal cut in front of this cuts off the orbital wing and extends to the edge of the cartilage. It is impossible to determine the anterior and posterior limits of the basisphenoid. The posterior limit is probably at or near the notochord and the anterior limit somewhere near the line (\*). The body is biconcave in the region of the sella turcica. Anteriorly, it narrows from side to side, as shown in figure 1, and increases in its sagittal thickness, as shown in figure 5. The temporal wing projects below the body and the orbital wing above. The mesethmoid

is shown intact after removal of the nasal capsule. The large crista galli and anterior end consist largely of young cartilage and precartilage.

#### INFERIOR ASPECT.

In comparing the inferior surface of the chondrocranium with that of the adult, only the occipital and otic regions lend themselves readily to our purpose, unless we imagine as eliminated the membranous bones, the maxillæ, the palatines, the vomer, the pterygoid process, most of the great wing of the sphenoid, and the tympanic part of the temporal. Figure 6 is drawn with the pharyngeal surface of the basioccipital parallel to the plane of the paper. This is not the usual view of the adult skull, and the latter must be rotated into the corresponding plane for comparison.

The basioccipital and the basisphenoid present no new features of especial interest not already considered, except the openings of the canal for the notochord. The anterior opening where the notochord reënters the basal plate is probably near or just back of the junction of the basioccipital and basisphenoid. The exoccipital shows the large hypoglossal foramina; these are not covered by the large condyles, as is the case in the adult. The condyles have scarcely begun to develop, and the occipital is united to the atlas by dense mesenchyme without articular surface. The tip of the occipital neural arch is quite distinct and separated from the squama. The prominent transverse or jugular process lies just caudal to the jugular foramen and, as in the adult, extends slightly lateral to the outer border of the foramen. The process is more prominent than in the adult and has more the character of the vertebral transverse processes.

The immature condition of the inferior surface of the otic capsule and mastoid region is accentuated by the fact that such structures as the carotid artery and the facial nerve are not inclosed by cartilage, but lie partly in sulci beneath the capsule. The internal carotid artery passes forward and medially in the mesenchyme beneath the cochlear part, with only the beginning of a groove formed by precartilage, not indicated in this figure. It enters the cranial cavity between the apex of the cochlear part and the alar process. The facial nerve, which enters the otic capsule through the internal acoustic meatus, soon emerges again from the facial foramen located in the anterior region of the capsular sulcus between the cochlear and canalicular parts. The geniculate ganglion lies just outside this foramen (figs. 12 and 13). The greater part of the facial nerve, which in the adult is inclosed in bone, is extracapsular and passes backward and outward in a groove on the under surface of the canalicular part. The groove lies between the fossa ineudis and the fenestra vestibuli or ovalis, and medial to the root of the styloid. The cartilaginous auditory ossicles lie beneath the canalicular part (fig. 14).

The serial relationship of the jugular process, the mastoid process, the upper end of the styloid, and the incus is clearly indicated in figure 6, in which the jugular, mastoid, root of the styloid, and fossa ineudis are shown. It is impossible to determine at present whether this has any phylogenetic significance or not. In some of the embryos of about this stage there is a small separate cartilage in the

blastema of the mastoid process to which are attached the digastric and stapedius muscles. The serial relationship shown in the embryo is quite different from that of the adult, where the mastoid process which gives attachment to the digastric is some little distance lateral to the jugular process, and the styloid process is immediately in front of the latter. Just in front of the jugular foramen is the opening for the aquæductus cochleæ.

The sphenoid shows the basisphenoid with the opening of the hypophysial canal near its center and the two wings on each side. Part of the orbital wing is obscured by the lateral part of the nasal capsule, as it would be in the adult from this point of view.

The line of fusion of the nasal capsule with the mesethmoid is shown, and the anterior nares directly face the observer as they do in the adult. The junction of the nasal capsules with the mesethmoid is effected along the middle third of the anterior edge of the mesethmoid by precartilage. The cut edge of this junction is shown in figure 5.

#### LATERAL ASPECT.

The cartilaginous skull covers but a small area of the lateral surface of the brain, namely, part of the medulla, part of the cerebellum, and a small area in the region of the optic nerve (figs. 7 and 8). The cartilaginous skull, even in its completed form, is a very inadequate protector for the brain, never covering more than a small fraction of its surface. The condensed mesenchyme or blastema covers a much larger surface, but even the cartilage and blastema together form at this stage a very incomplete brain capsule (fig. 9). The capsule is completed by the thin dorsal membrane. The inclosing of the central nervous system by the gradual spreading of the blastema and cartilage, which invade and replace the dorsal membrane, is similar to the well-known development of the thoracic and abdominal walls and the disappearance of the ventral membrane.

The blastema covers almost all of the lateral surface of the cartilaginous skull. A small part of the occipital cartilage, including the transverse process, part of the squama and occipital neural arch, part of the orbital wing of the sphenoid, and part of the lateral surface of the nasal capsule, are uncovered (figs. 9 and 15). Into the blastema covering the squamal cartilage, rather than into the cartilage itself, are inserted the various occipital muscles (figs. 14 and 15). The blastema covering the squama and the lateral surface of the otic capsule probably fuses later with the perichondrium, but at this stage it seems to be continuous with the rest of the blastemal wall which later gives rise to membrane bones. It is in the sphenoidal and frontal regions that the blastema greatly predominates over the cartilage. All of the lateral wall of the middle cranial fossa consists of blastema and the greater part of the floor (as well as all of the lateral wall of the anterior fossa) is likewise formed by blastema. The orbital walls are mostly of blastema; cartilage of the orbital wing of the sphenoid takes part in the formation of the apical region of the orbit about the optic foramen, and a portion of the medial wall of the orbit is formed by part of the lateral wall of the nasal capsule. Connected with the



cartilage is considerable precartilage extending along the inner wall towards the apex (fig. 15). Part of the outer wall of the orbit, consisting of the zygomatic blastema and the zygomatic process of the frontal blastema, and also the zygomatic arch, are shown in figure 9. In lateral views of the adult skull the upper border of the zygomatic arch is horizontal, and it is therefore easier to compare figure 9 and the other lateral views with the adult if the figures are rotated so as to bring the zygomatic arch into a horizontal position. Attention has already been called to the fact that the angle made by projecting the line of the upper edge of the zygomatic arch on to the basioccipital is almost exactly the same in the embryo and in the adult.

By rotating figure 7 to correspond to the usual adult position it will be seen that the occipital squama projects backward from the exoccipital and mastoid, and that, since it covers only part of the medulla and cerebellum, it is concerned with the wall of the posterior cranial fossa. It is from this lateral view that one gets the impression that the squama extends into the jugular process, or more strictly, into that part of the exoccipital which continues into the lateral part of the jugular process, rather than into the occipital neural arch which lies in a deeper plane. The occipital neural arch has attained only a small proportion of the growth necessary to inclose the medulla. The tip of the squama, on the other hand, has grown farther around. The condition here is not as far advanced as that found by Levi in a supposedly younger embryo.

The lateral aspect of the jugular process is quite prominent, and to it are attached two muscles, the rectus capitis lateralis (serially related to the intertransversarii) and the occipitomastoid muscle (fig. 14). The former needs no special comment. The latter muscle is not found in the adult and is infrequent in embryos. Its presence would seem to indicate either that the mastoid process was separate or movable, or that the temporal and occipital cartilages were at one time movable on each other. We do find that the occipital and mastoid cartilages are separated by young cartilage or precartilage in the region below the mastoid foramen. Above this, however, no line of separation can be found, but this does not necessarily mean that the two cartilages were articulated by a movable joint. The significance of the mastoid process is described elsewhere.

The canalicular part of the otic capsule forms a conspicuous part of the lateral wall of the chondrocranium. A slight bulging on the lower part of its lateral surface indicates the position of the lateral semicircular canal. The styloid process and incus lie beneath the lateral edge of the otic capsule almost flush with the lateral surface.

Between the canalicular part and the occipital cartilage is the mastoid cartilage. It is fairly clearly defined in the region between the otic capsule and the exoccipital below the mastoid foramen. In the region above the otic capsule there is no line of demarcation between the squama and mastoid. The mastoid process projects from the lower edge of the mastoid cartilage. In this embryo it consists of blastema; in other embryos, as will be stated farther on, a small cartilaginous nodule is sometimes embedded in the blastema. To the mastoid process are



attached several muscles, namely, the occipitomastoid, the atlantomastoid, the digastric, the stapedius, and the longissimus capitis (figs. 14 and 15).

The anterior part of the cartilaginous skull; the sphenoid and the ethmoid, bears very little resemblance to anything ordinarily seen in the lateral view of the adult skull, partly because most of the bone ossified in cartilage becomes covered or shut out from the lateral view by membrane bone. If we can imagine all of this membrane bone stripped away, the resemblance between the two would be clearly seen.

The lateral surface of the body of the sphenoid, with the shallow sella turcica odging the hypophysis, is to be seen in front of the apex of the cochlear part of the otic capsule. The prominent dorsum sellæ projects towards the great mid-brain fissure, and from it (but not shown) the thin medial membranous part of the tentorium projects into this fissure. The temporal wing lies for the most part below the level of the basisphenoid and is attached to the under surface of the alar process, while the orbital wing lies, for the most part, above the level of the basisphenoid and is curved around the optic nerve. The two parts of the orbital wing are clearly indicated in figures 7 and 14. In the description of the middle cranial fossa and the embryonic tentorium cerebelli it was noted that the lateral prismatic mass was so placed that the apical edge extended upward from the dorsum sellæ in connection with the thin medial membranous part of the tentorium lying in the great mid-brain fissure. The lower end of the embryonic tentorium, as previously noted, occupies the space between the otic capsule and the orbital wing of the sphenoid, the semilunar ganglion filling up most of the gap (fig. 8). It lies outside of the cartilaginous skull but within the membranous skull. The geniculate ganglion and adjacent sections of the facial nerve are not incased in cartilage, and they also lie in this middle cranial fossa or gap (figs. 12 and 13).

In the lateral aspect of the chondrocranium most of the mesethmoid is shut out from view by the nasal capsule. Between the orbital wing and the nasal capsule the anterior end of the basisphenoid and a bit of the mesethmoid appear. If only the cartilaginous skull were considered, this region of the basisphenoid might be regarded as interorbital and the term *interorbital septum* could be applied. But this so-called interorbital septum exists only in the cartilaginous skull; in the more complete skull, with all the blastema and precartilage, the medial wall of the orbit is complete enough to shut it out from the orbit. The term *interorbital septum* probably has no significance whatever in human embryology. The prominent crista galli projects between the cerebral hemispheres. The lateral surface of the nasal capsule forms a large proportion of the inner wall of the orbit and probably ossifies into the lamina papyracea of the ethmoid.

#### THE OCCIPITAL CARTILAGE.

The occipital cartilage consists of an elongated, flattened body (or basioecipital), the lateral parts (or exoccipitals), and the two nuchal plates (or squamæ).

The body forms the greater part of the so-called basal plate, a term much used in descriptions of the cartilaginous skull. It continues without line of demarcation

into the basisphenoid, and one can only approximate the future line of junction between the two bones which ossify in the cartilage by the relation to the apex of the otic capsule and the entrance of the notochord. The basioccipital lies in a plane almost exactly horizontal to the long axis of the embryo. This elongated quadrilateral plate is thinner at the center, slightly concave on the cranial surface, and slightly convex on the pharyngeal surface. It becomes thicker where it joins the sphenoid. The rounded caudal end is bent downward and notched in the midline at the foramen magnum. The caudal part of the basioccipital gives much more of a clue to its vertebral origin than the flattened cranial part. The caudal part is thickened and on the cranial surface is incompletely divided into two lateral masses by a deep median groove. These correspond, perhaps, to the bilateral masses which fuse to form the bodies of the cervical vertebrae. Levi finds in his 13 mm. embryo two bilateral cartilaginous centers medial to the hypoglossal roots. They probably represent, as Macklin suggests, the bilateral centers for the occipital vertebrae. Their position in the 13 mm. embryo corresponds to the position of the lateral masses in this 21 mm. embryo. These masses are united across the midline beneath the notochord, and anteriorly are continued into the broad flat plate of the basioccipital. The center of each mass contains the most highly differentiated cartilage of the entire chondrocranium.

In the 14 mm. embryo described by Levi the otic capsule and the basioccipital are apparently separated by a wide gap. Levi probably did not include all the precartilaginous tissue and blastema of the otic capsule, since in embryo No. 109 (Carnegie Collection), 11.5 mm. in length, the blastema of the otic capsule is in contact with the basioccipital. It is only in younger stages, 9 mm. length, that I find the blastema of the otic capsule separated from the basioccipital. The union of the blastema, then, precedes that of the cartilage in this region. In embryo No. 460 the anterior half of the lateral border of the basioccipital is fused with the cochlear part of the otic capsule along a crescentic line. The line of fusion can still be recognized by the differences in the degree of differentiation of the two cartilages. The cochlear part consists of young cartilage and the nuclei are closer together than in the basioccipital. The border of the basioccipital is, however, not so far advanced as the central part. The two cartilages were so completely fused in the 17 mm. embryo described by Levi that he was unable to recognize any histological border between them. Embryo No. 128 (Carnegie Collection), 20 mm. in length, although supposedly smaller and younger than the 21 mm. embryo (No. 460), shows a more advanced condition in the cartilaginous differentiation of this region, and it is impossible to find a histological border between the basioccipital and the cochlear part except at either end of the line of fusion. In another 20 mm. embryo (No. 22, Carnegie Collection) there is only the very slightest indication of the line of fusion. There is probably some variation in the rate of differentiation of the cartilages of this region in embryos of the same age and size.

## OCCIPITAL VERTEBRA.

Each exoccipital corresponds for the most part to a vertebral neural arch or hemiarch, which is so distinct in this embryo that we may properly apply the term *occipital hemiarch*. The roots, laminae, and transverse processes can be readily recognized. The roots are broad and thick and correspond to the roots or pedicles of the vertebral neural arches. On the right side, as already noted, there are three roots dividing the cranial end of the hypoglossal canal into two parts, a condition not uncommon in the adult. The caudal root on each side is the largest and consists of more advanced cartilage, and it is questionable whether the anterior roots should be looked upon as forming part of the occipital vertebra. It has often been suggested that they are part of an anterior, still more rudimentary vertebra. The laminae extend outward and upward from the roots, forming part of the lateral border of the foramen magnum. They are much thickened, rounded, tapering cartilages, quite distinct, and in the dorsal part entirely separate in this particular embryo from the squamal cartilage. The cartilage of the occipital arch is more differentiated than the squamal. During the further course of development, as Macklin has pointed out, the occipital neural hemiarches grow around the central nervous system and approach each other in the midline. It is not clear whether they actually meet and fuse, or whether a small part of the squamal cartilage may not intervene. The squamal cartilages, which likewise grow around the central nervous system, meet and fuse before the occipital hemiarches. The ossicle of Kerckring, which develops in this region, may represent a separate ossification center of the nuchal plate intervening between the tips of the occipital neural hemiarches, or this center may possibly be looked upon as the ossification of the fused occipital spinal epiphyses. Schultz described a skull with two bilateral ossicles in this region, and suggests that they may correspond to the epiphyses of the spinous processes.

In this embryo the separation of the dorsal part of the lamina of the occipital vertebra from the squama of the occipital, and the more intimate relation of the latter with the transverse process, would seem to indicate that the squama is an extension upward from the transverse process rather than an outgrowth from the occipital neural lamina. The relation of the squama to the transverse process and to the occipital neural lamina is clearly shown in figures 1, 7, and 16. The occipital transverse process forms part of the caudal and lateral margins of the jugular foramen and continues up into the squama and alar lamina without line of demarcation. The alar lamina, as will be seen in figure 16, constitutes the ventral and more medial part of the squama adjoining the lamina of the occipital and the medial part of the transverse process. There is also a more gradual transition as regards the degree of differentiation from the cartilage of the transverse process into the squama than from that of the lamina.

In embryo No. 22 (Carnegie Collection), 20 mm. in length, the squama of the occipital is fused with the lamina throughout the entire length of the latter on both sides of the embryo, although throughout most of this length the younger cartilage of the squama can easily be distinguished from the thickened, older cartilage of the lamina. Towards the apex of the arch, however, the two cartilages

are of the same degree of differentiation and no distinction between them can be seen.

In embryo No. 128 (Carnegie Collection), 20 mm. in length, the resemblance which the occipital neural arch and its transverse process bear to those in the cervical region is very striking. The cartilages of the occipital and cervical neural arches show much more advanced differentiation than the thin transverse processes. The muscle *intertransversarius*, between the transverse processes of the second and the first cervical vertebræ, is in line with and serially related to the *rectus capitis lateralis*, which passes between the transverse process of the first cervical vertebra and that of the occipital. In this embryo (No. 128) it can be seen also how the squama of the occipital is a continuation upward from the transverse process rather than from the lamina. The laminae of the occipital vertebra are also fused along the entire length to the squamæ, but are readily distinguishable from the latter by their much more advanced cartilaginous differentiation. The tips of these laminae are connected with the tips of the laminae of the atlas by the interspinous ligament, as in embryo No. 460.

In embryo No. 240 (Carnegie Collection), 20 mm. in length, the cartilage of the occipital neural arch on each side is likewise fused with the squama throughout its entire length. The lamina also shows more advanced differentiation than the squama. In embryo No. 431 (Carnegie Collection), 19 mm. in length, the tips of the occipital neural arches are separated from the squama on either side by the condensed mesenchyme forming the perichondrium. The extent of the fusion of the laminae and the squamæ is, however, more extensive than in embryo No. 460. These observations indicate that the occipital neural arch is more marked in this stage than in the adult, and that probably an extreme degree of accentuation in the embryo would precede any marked manifestation of an occipital vertebra in the adult. The manifestation of an occipital vertebra in adult skulls has been described by Gladstone, Kollman, and others. Such variations remind one of the conditions found in embryos at this stage, and we shall probably find that embryos vary as much, if not more, than adults and that such embryonic variations always precede the adult variations.

The relation and attachment of the dorsal membrane to the tips of the occipital hemiarches are similar to its relation and attachment to the tips of the neural arches of the cervical vertebræ, in that in both cases the dorsal membrane is continuous with the perichondrium on the medial side of the tip of the neural arch. Its attachment to the upper border of the squama is somewhat different, since here it is merely continuous with the thin edge. The tip of the occipital hemiarch is connected with the tips of the vertebral hemiarches by a distinct band of condensed membrane, the interspinous ligament described by Bardeen (fig. 9).

The transverse or jugular process springs from the occipital hemiarch at the junction of the roots and lamina and projects laterally back of the jugular foramen. We have already noted its serial relationship with the vertebral transverse processes. The lateral extremity of the jugular process has a knob-like enlargement, and into this are inserted the *rectus capitis lateralis* muscle and the occipito-mastoid muscle (fig. 24).

The occipital squama or nuchal plate springs from the upper border of the lateral part of the jugular process. Near its origin it is narrow in the sagittal plane and wide in the frontal plane. It is continuous with the basal part of the lamina by a curved plate, the alar lamina (figs. 1 and 16), and rapidly widens into a broad, thin plate, which continues upward to a thin edge that runs into the dorsal membrane. Both the jugular process and the squama extend into the mastoid cartilage. At the jugular foramen these two cartilages are separated by blastema and precartilage; above this they are joined by young cartilage, which gradually changes into cartilage indistinguishable from that of the squama or mastoid. The lateral surface of the squama is covered by condensed mesenchyme into which the edges of the cartilage merge. It is into this mesenchyme that the occipital muscles—namely, the trapezius, splenius capitis, semispinalis, rectus capitis posticus major and minor, and the obliquus capitis superior, appear to be inserted (figs. 14 and 15).

#### THE SPHENOID CARTILAGE.

The sphenoid cartilage consists of a body with two lateral wings or processes on each side, the temporal and orbital wings (figs. 1, 2, 3, and 6). The cartilage of the body is continuous with the body of the occipital, and in front narrows and thickens as it passes into the nasal septum. The cartilaginous center from which the body develops is described as being caudal to the hypophysial canal. The chondrification spreads caudally to meet the basioccipital, upward into the dorsum sellæ, and forward around the hypophysial canal where the cartilage fuses around the canal and extends forward to form the anterior part of the sphenoid and the nasal septum. Fawcett, after an examination of a 21 and a 19 mm. embryo, concluded that the dorsum sellæ was an independent formation, since he found in each embryo a separate cartilaginous bar above the region of the body and separated from it by mesenchyme. Twenty embryos of the Carnegie Collection, ranging in length from 15.5 mm. to 24 mm., were examined, and such a cartilaginous bar was found in but one (No. 229), 19 mm. long. Twelve of the embryos were between 18 and 23 mm. Great variation in the form of the dorsum sellæ was observed, but the rather rare presence of a separate center of chondrification would indicate that as a general rule the dorsum sellæ develops from the basisphenoid, as described by Levi. In front of the dorsum sellæ the body is biconcave and perforated near the center by the hypophysial canal. There is practically no indication in front of the sella turcica of the tuberculum sellæ. The anterior part of the body is very incompletely developed, and not until the anterior tip of the orbital wing has grown medialward to meet its fellow of the opposite side and fuses with the body is the anterior border of the chiasmatic groove apparent (fig. 3).

The temporal wing consists of two parts: the alar process, which springs from the body just in front of the level of the dorsum sellæ and projects laterally and slightly caudally toward the apex of the cochlear part of the otic capsule, and the lateral part, attached to the under surface of the alar process and projecting laterally. Each part is supposed to have an independent center of chondrification. In this embryo the alar process is joined to the basisphenoid by young cartilage and

to the lateral part by blastema. We have already given sufficient consideration to the fate of the alar process and its probable incorporation into the body of the sphenoid, and also the lateral part which corresponds more strictly to that part of the temporal wing that ossifies in cartilage. This part of the cartilage is still incompletely developed; as in later stages the cartilage extends around the maxillary nerve.

The orbital wing is even more immature than the temporal, as will be seen by comparing it with the older stages of Macklin and Hertwig. In figure 3 is shown the precartilaginous part, which is more extensive than the cartilaginous part. The basal part of the orbital wing lies at the apex of the orbit and gives attachment to all the muscles of the orbit except the superior oblique (figs. 10 and 15).

#### THE TEMPORAL CARTILAGE.

This cartilage includes the otic capsule and the mastoid cartilage, which are intimately fused together.

##### OTIC CAPSULES.

The otic capsules form the cartilaginous basis for the petrous bones of the adult. They are very prominent, being striking features of the chondrocranium, and extend on each side in a caudo-dorso-lateral direction to the lateral surfaces of the skull. Each capsule consists of two broadly united and continuous parts (a medial cochlear part and a lateral canalicular part) inclosing the cochlear and semicircular canals respectively. The two parts are more or less set off from each other by a broad shallow groove, the capsular sulcus.

The cochlear part is somewhat egg-shaped and fuses with the basioccipital. The cranial surface is rounded and presents the large, rounded, internal acoustic meatus. The ventral surface is also rounded and projects below the level of the basioccipital, forming with it a distinct groove. The cochlear part consists of somewhat younger cartilage than the canalicular part. Its relation to the cochlear duct is shown in figure 16.

The capsular sulcus extends entirely around the otic capsule. On its anterior surface is a large foramen for the exit of the facial nerve. This foramen is separated by a narrow bar of cartilage from the internal auditory meatus. The facial nerve has but a very short course within the otic capsule; the geniculate ganglion and the nerve distal to it are outside of the capsule, but close against it (figs. 12 and 13). The capsular sulcus is broad and shallow on the inferior surface and near its center lies the fossa vestibularis or ovalis, in which is imbedded the base of the stapes. Immediately about the fossa is an area of young cartilage; the floor of the fossa is also covered with young cartilage. The posterior part of the sulcus, which borders the jugular foramen, contains the opening for the aqueductus cochlearis. At this stage only a small plexus of veins pass through it, but later it contains the perilymphatic duct.

The canalicular part of the otic capsule constitutes the lateral half and forms part of the outer wall of the chondrocranium. It is more or less oval in form and flattened in a medio-lateral direction. It consists for the most part of the thickened cartilaginous covering for the semicircular ducts. The locations of

the latter are more or less apparent by the configuration of the cartilage. The position of the superior semicircular duct is quite clearly indicated; it lies within the prominent, rounded semicircular mass constituting the antero-dorsal portion of the canalicular half of the capsule (figs. 1, 2, 3, 5 and 15). The deep fossa subarcuata lies at the center of its curve. The lateral semicircular canal produces a distinct bulge on the lower part of the lateral surface of the capsule, while the position of the posterior duct is indicated by the configuration of the posterior portion of the canalicular part. The cranial surface shows a well marked sulcus for the endolymphatic duct which leads into the opening for the aqueductus vestibuli. The upper edge of the cartilage, where it joins the mastoid, is deeply grooved for the transverse sinus. The lower edge of this posterior portion projects ventrally in the form of a ridge, the crista parotica, to the caudal end of which is attached the styloid process. In front of the crista there is a slight depression, the fossa incudis, in which is lodged the short crus of the incus. The facial nerve lies in a groove just medial to the crista and styloid process (figs. 6, 12, and 13).

#### MASTOID CARTILAGE.

The mastoid cartilage is continuous with the caudal and upper border of the canalicular part. We have already described its fusion with the exoccipital and squama of the occipital. Its inner surface is hollowed out for the great transverse sinus, and in the upper part of this sulcus is the large mastoid foramen. A distinct mass of blastema or precartilage projects ventrally from the inferior edge of the mastoid just back of the root of the styloid process. Since to this mass are attached the digastric, splenius capitis, longissimus capitis, and sternomastoid muscles, it probably represents the mastoid process. In addition to the above-mentioned muscles the stapedius also appears to arise from the inner side of the mass. At this stage the stapedius is relatively large and entirely extracapsular. Two interesting rudimentary muscles, not found in the adult, are likewise attached to this mastoid process. One, short and thick, arises from the occipital transverse process; the other, longer and more slender, extends from the transverse process of the atlas (figs. 14 and 15). I have named these muscles the *occipito-mastoid* and the *atlanto-mastoid*. The occipito-mastoid is serially related to the intertransversarii.

This mastoid blastema, or mastoid-process blastema, presents interesting variations. In embryo No. 382 (Carnegie Collection), 20 mm. in length, there is a small nodule of cartilage in this region—the mastoid-process cartilage. This is attached to the occipital transverse process by a short ligament. Both the digastric and stapedius muscles are attached to it, but no trace of the occipito-mastoid or atlanto-mastoid muscles were found. In embryo No. 431 (Carnegie Collection), 19 mm. in length, a similar nodule of young cartilage was found attached in the same manner to the transverse process of the occipital by a ligament. The stapedius muscle is attached to its inner surface and the digastric to its lower surface. In both embryos the mastoid-process cartilage is closely associated with the mastoid blastema and the ligament or band of condensed mesenchyme, connecting it with the jugular process, occupies the same position as the occipito-mastoid muscle found in embryo No. 460. The attachment of the stapedius and digastric



suggests that the mastoid process may be regarded as a remnant of the proximal end of a branchial cartilage, while its serial relation with the transverse process of the occipital and cervical vertebræ, and its connection with the former by muscle, as in embryo No. 460, or by ligament, as in embryos Nos. 431 and 382, suggest that it may be a rudimentary portion of the transverse process of an occipital or temporal vertebra. A still more speculative idea would be to consider the branchial bars as serially related to the vertebral transverse process, and the occipito-mastoid muscle could be looked upon as serially related to the intertransversarii muscles on the one hand and to the stapedius on the other.

#### THE ETHMOID CARTILAGE.

The ethmoid cartilage consists of the mesethmoid and the lateral nasal capsules joined to the former by precartilage. The ethmoid is very poorly developed at this stage and all the figures include more or less precartilage. The gradual transition from cartilage to young cartilage and precartilage makes it impossible to draw sharp lines between the cartilage and precartilage. The mesethmoid consists of fairly well-differentiated cartilage near its junction with the sphenoid. In this region the pharyngeal edge is much thicker than the dorsal edge. It gradually tapers towards the apex, the entire septum attaining an even thickness. The crista galli is relatively large when both cartilage and precartilage are included, as shown in the various figures.

The nasal capsules consist mostly of young cartilage and precartilage. They are relatively simple and show only slight indications of the turbinate processes. Part of the lateral surface enters into the formation of the medial wall of the orbit and part is covered by maxillary and nasal blastema, the precartilaginous tips extending beyond (fig. 15). The inner wall of the orbit is completed by frontal blastema and precartilage of the orbital wing of the sphenoid. This blastema and precartilage, as already noted, form a continuous sheet (fig. 3). The orbital surface of the sheet is more or less closely united to the upper edge of the nasal capsule (fig. 15). On the line of junction between the cartilage and frontal blastema are found, as in the adult, the anterior and posterior ethmoidal foramina, through which pass the nasociliary nerve and the ethmoidal branches.



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EXPLANATION OF FIGURES.

- FIG. 1. Dorsal aspect of base of the cartilaginous skull with the basioccipital in the horizontal plane. The cervical vertebrae are included. At the anterior end some precartilag. is included with the crista galli and the nasal capsule.  $\times 10$  diameters.
- FIG. 2. Right half of dorsal aspect of the base of the cartilaginous skull. The adult position, basioccipital inclined at an angle with the horizontal plane.  $\times 10$  diameters.
- FIG. 3. Dorsal aspect of cartilaginous and membranous skull. Same view as shown in figure 1.  $\times 10$  diameters.
- FIG. 4. Dorsal aspect of base of the adult skull. Bone ossified in cartilage stained blue. Modified from Spalteholz Atlas.
- FIG. 5. Median sagittal aspect of the cartilaginous skull. Part of crista galli and anterior end of mesethmoid are precartilag.  $\times 10$  diameters.
- FIG. 6. Ventral aspect of base of the cartilaginous skull.  $\times 10$  diameters.
- FIG. 7. Lateral aspect of cartilaginous skull and cervical vertebrae, with the brain and cervical cord and hypophysis in position. Merkel's cartilage, the styloid, hyoid, and laryngeal cartilages are also shown.  $\times 10$  diameters.
- FIG. 8. Lateral aspect of cartilaginous skull and cervical vertebrae with the brain, cervical cord, and nerves.  $\times 10$  diameters.
- FIG. 9. Lateral view of cartilaginous skull and cervical vertebrae with the overlying membranous skull and the dorsal membrane.  $\times 10$  diameters.
- FIG. 10. Dorsal aspect of sphenoid cartilage, showing attachment of the orbital muscles to the basal part of the orbital wing.  $\times 20$  diameters.
- FIG. 11. Dorsal aspect of sphenoid cartilage.  $\times 20$  diameters.
- FIG. 12. Lateral view of the right otic region. Part of the malleus and incus cut away showing course of facial nerve and position of otic ganglion.  $\times 20$  diameters.
- FIG. 13. Lateral view of right otic region showing relations of facial nerve.  $\times 20$  diameters.
- FIG. 14. Lateral view of base of cartilaginous skull with deeper muscles of occipital region and of the mouth and pharynx.  $\times 20$  diameters.
- FIG. 15. Lateral view of part of cartilaginous and membranous skull. The inner wall of the orbit and part of occipito-temporal region exposed by cutting away part of the membranous skull.  $\times 20$  diameters.
- FIG. 16. Dorsal view of temporal and occipital cartilages, showing the relation of the inner ear to the otic capsule.  $\times 20$  diameters.

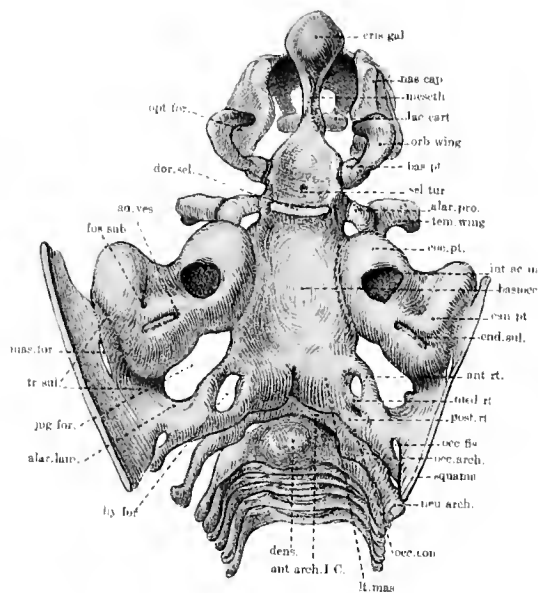
All of the figures except No. 4 are from models or combinations of models and graphic reconstructions of one embryo No. 460, Carnegie Collection.

Cartilage is colored blue, except figure 4, precartilag. green, nerves yellow, muscles red; the blastema remains uncolored.

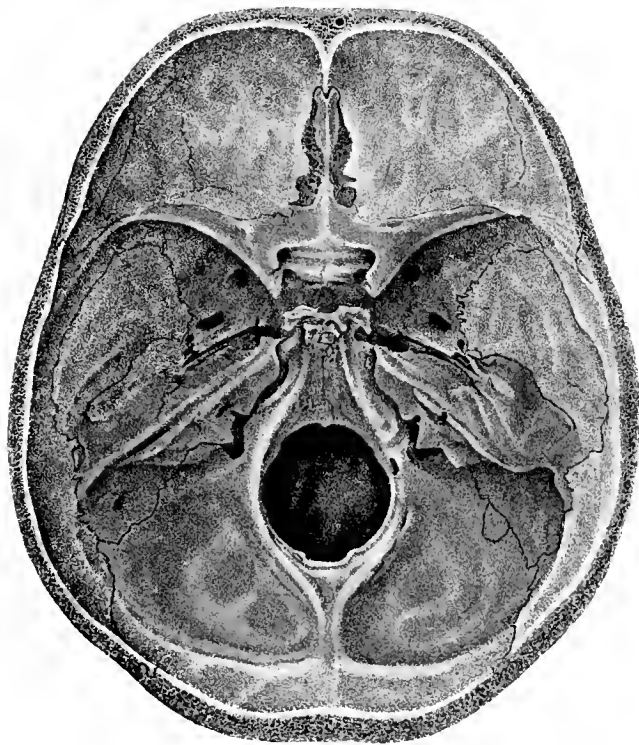
The illustrations are mainly the work of Mr. C. W. Shepard and Mr. J. F. Didusch.

## ABBREVIATIONS.

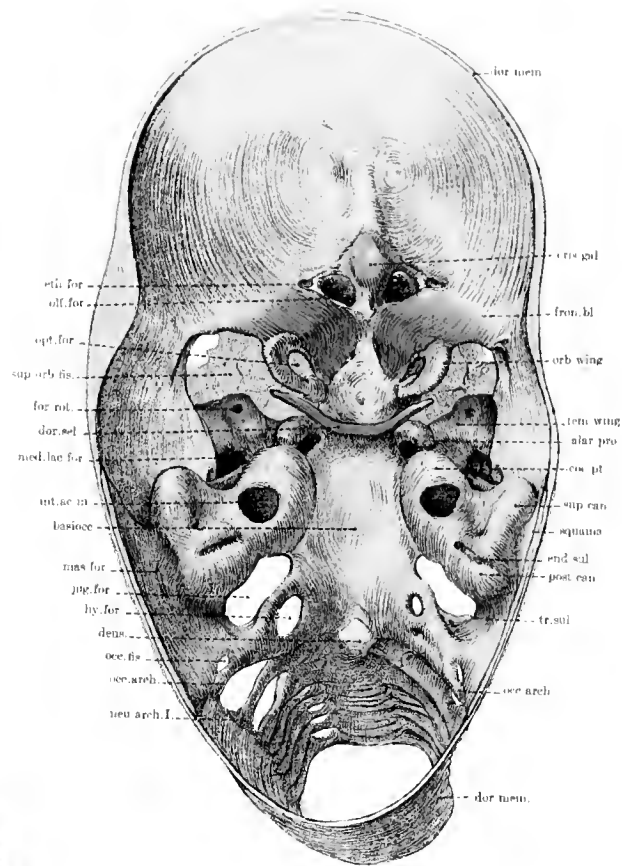
alar. lam.,	alar lamina	max.,	maxillary blastema.
alar. pro.,	alar process.	med. lac. for.,	median lacerated foramen.
amp. lat. d.,	ampulla lateral semicircular duct.	med. rt.,	median root occipital neural arch.
amp. p. d.,	ampulla posterior semicircular duct.	Meek.,	Meekel's cartilage.
amp. s. d.,	ampulla superior semicircular duct.	meseth.,	mesethmoid.
ansa. hy.,	ansa hypoglossi.	nas. bl.,	nasal blastema.
ant. arch. I. C.,	anterior arch atlas.	nas. cap.,	nasal capsule.
ant. nar.,	anterior nares.	neu. arch.,	neural arch.
ant. rt.,	anterior root occipital neural arch.	obl. cap. inf.,	obliquus capitis inferior muscle.
aq. ves.,	aqueduct vestibuli.	obl. cap. sup.,	obliquus capitis superior muscle.
atl. mas.,	atlanto-mastoid muscle.	occ. arch.,	occipital neural arch.
atl. occ. art.,	atlanto-occipital articulation.	occ. fis.,	occipital fissure.
basiocc.,	basiooccipital.	occ. mas.,	occipitomastoid muscle.
bas. pt.,	basal part orbital wing sphenoid.	occ. con.,	occipital condyle.
biventer,	biventer cervicis muscle.	olf. for.,	olfactory foramen.
can. pt.,	canalicular part otic capsule.	opt. for.,	optic foramen.
chr. pl.,	choroid plexus.	opt. n.,	optic nerve.
ch. ty.,	chordatympani.	orb. wing,	orbital wing.
cil. g.,	ciliary ganglion.	ot. cap.,	otic capsule.
coe. d.,	cochlear duct.	otic gang.,	otic ganglion.
coe. pt.,	cochlear part otic capsule.	pal. n.,	palatine nerve.
com. d.,	common duct.	para. gl.,	parathyroid gland.
cris. gal.,	crista galli.	post. rt.,	posterior root occipital neural arch.
digas.,	digastric muscle.	rec. cap. ant.,	rectus capitis anterior muscle.
dor. mem.,	dorsal membrane.	rec. cap. lat.,	rectus capitis lateralis muscle.
dor. sel.,	dorsum sellae.	rec. cap. post.,	rectus capitis posterior muscle.
ed. bl.,	edge cranial blastema.	sel. tur.,	sella turcica.
end. d.,	endolymphatic duct.	semispi. cap.,	semispinalis capitis muscle.
end. sul.,	endolymphatic sulcus.	sem. g.,	semilunar ganglion.
epiph.,	epiphysis.	squama,	squama occipital cartilage.
eth. for.,	ethmoid foramen.	sph. pal. g.,	sphenopalatine ganglion.
fac. for.,	facial foramen.	sph. pal. n.,	sphenopalatine nerve.
fac. sul.,	sulcus for facial nerve.	ster. thy.,	sternothyroid muscle.
fen. ves.,	fenestra vestibuli.	sty. glo.,	styloglossus muscle.
for. rot.,	foramen rotundum.	sty. hy.,	stylohyoid muscle.
fos. inc.,	fossa incudis.	sty. ph.,	stylopharyngeus muscle.
fos. sub.,	fossa subarcuata.	submax. cap.,	submaxillary gland capsule.
fron. bl.,	frontal blastema.	sup. can.,	superior semicircular canal.
gang. I. C.,	ganglion first cervical nerve.	sup. ling.,	superior lingual muscle.
gen. g.,	geniculate ganglion.	sup. orb. fis.,	superior orbital fissure.
gen. glo.,	genioglossus muscle.	sup. rec.,	superior rectus muscle.
gen. hy.,	geniohyoid muscle.	temp. wing,	temporal wing.
gr. sup. pet. n.,	greater superficial petrosal nerve.	thy. hy.,	thyrohyoid muscle.
hy. for.,	hypoglossal foramen.	thym. gl.,	thymus gland.
hyo. glo.,	hyoglossus muscle.	thyr.,	thyroid cartilage.
hyp. c.,	hypophyseal canal.	thyr. gl.,	thyroid gland.
hyp.,	hypophysis.	tr. pro.,	transverse process atlas.
inf. alv.,	inferior alveolar nerve.	tr. sul.,	transverse sulcus.
inf. col.,	inferior colliculus.	zyg.,	zygoma blastema.
inf. orb.,	infraorbital nerve.	zyg. arc.,	zygomatic arch.
in. sp. lig.,	interspinous ligament.	zyg. pro.,	zygomatic process of frontal blastema.
int. ac. m.,	internal acoustic meatus.		
Jac. cart.,	Jacobson's cartilage.	III.	III. cranial nerve.
jug. for.,	jugular foramen.	IV.	IV. " "
jug. pro.,	jugular process.	V.	V. " "
jug. v.,	jugular vein.	VI.	VI. " "
lat. rec.,	lateral rectus muscle.	VII.	VII. " "
lig. fla.,	ligamenta subflava.	IX.	IX. " "
lon. cap.,	longissimus capitis muscle.	X. }	X. } " "
lt. mas.,	lateral mass basiooccipital.	XI. }	XI. } " "
mand.,	mandible.	XII.	XII. " "
mas. for.,	mastoid foramen.	I. C. N.	I. cervical "
mas. pro.,	mastoid process.	II. C. N.	II. " "
		*	line between sphenoid and mesethmoid



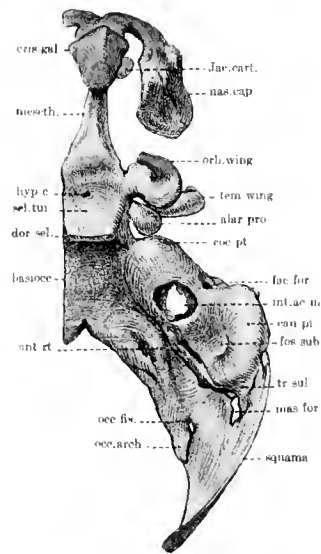
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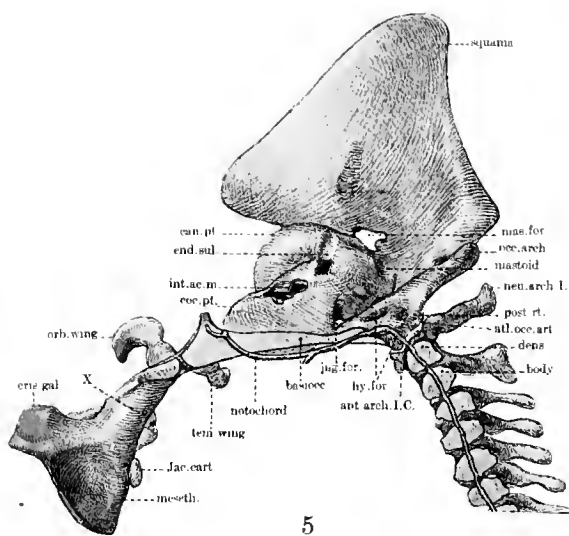


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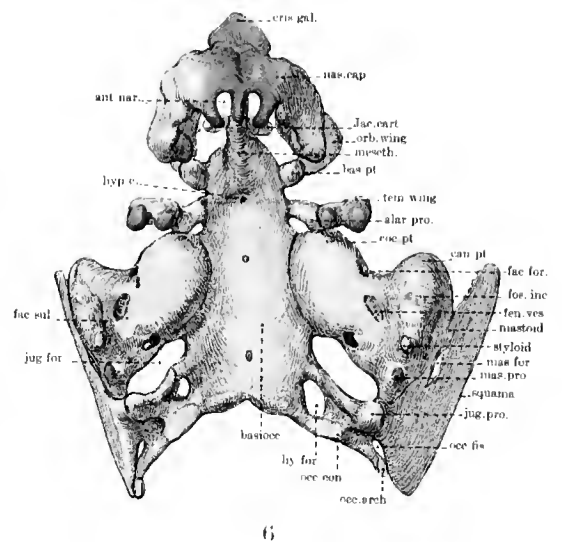


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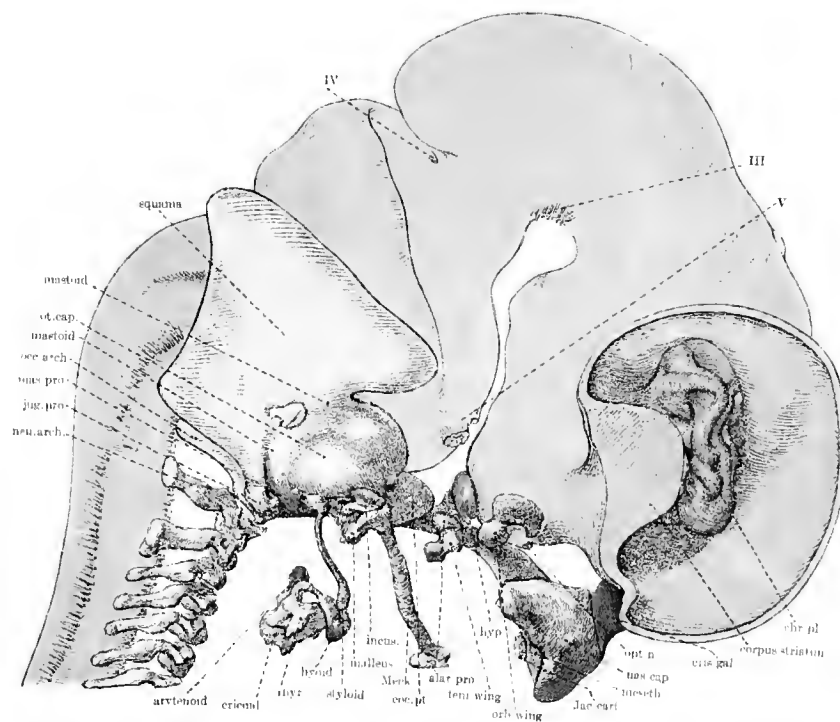




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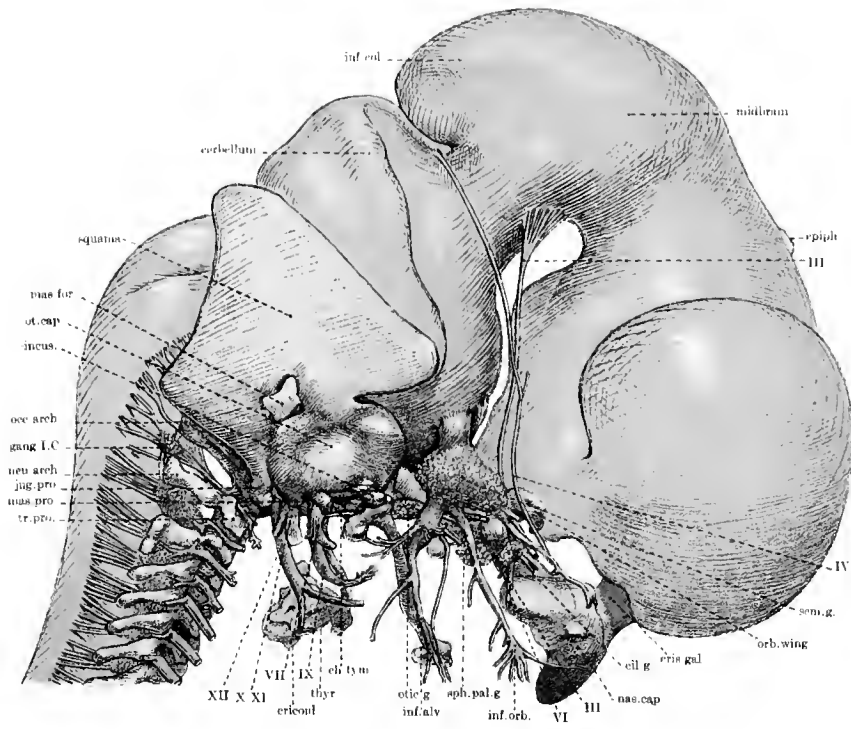


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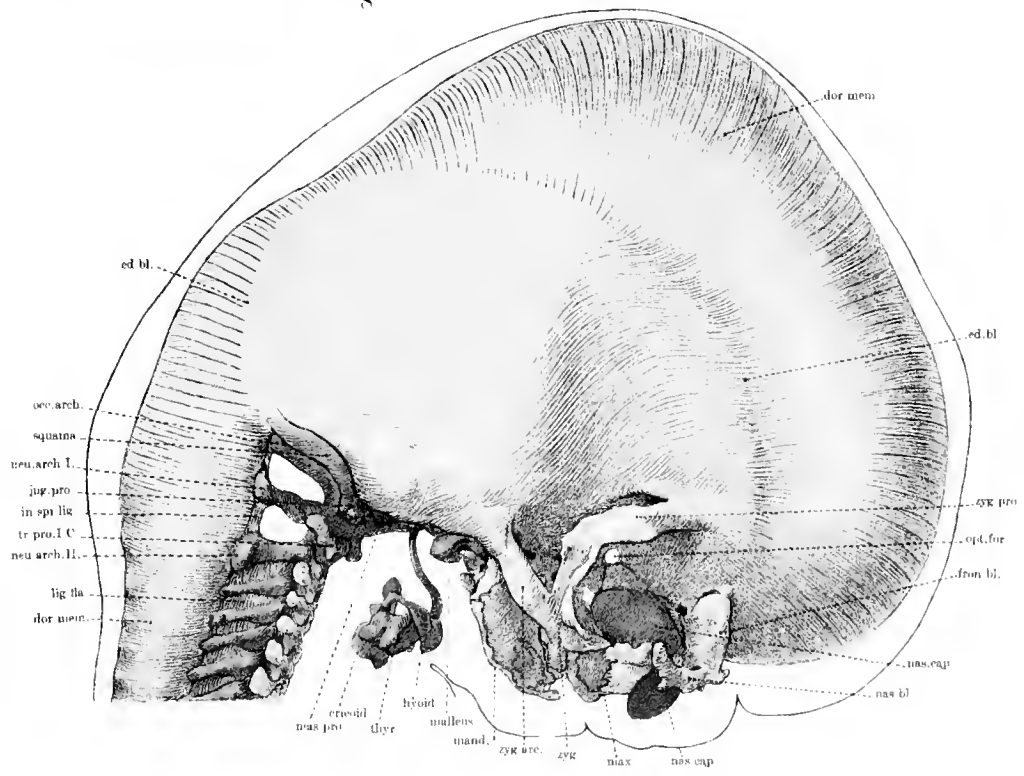


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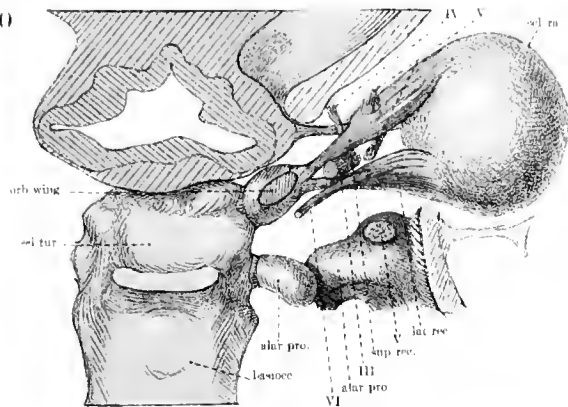


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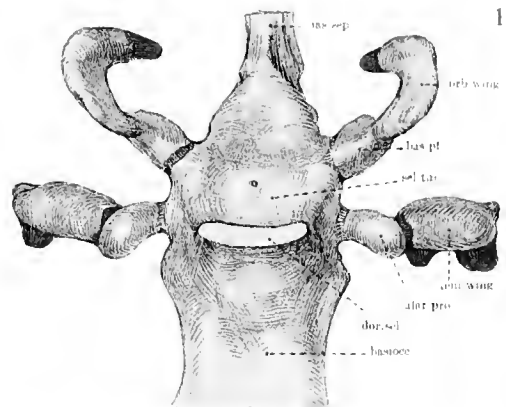




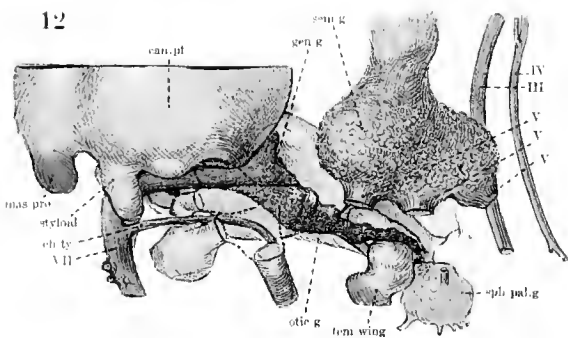
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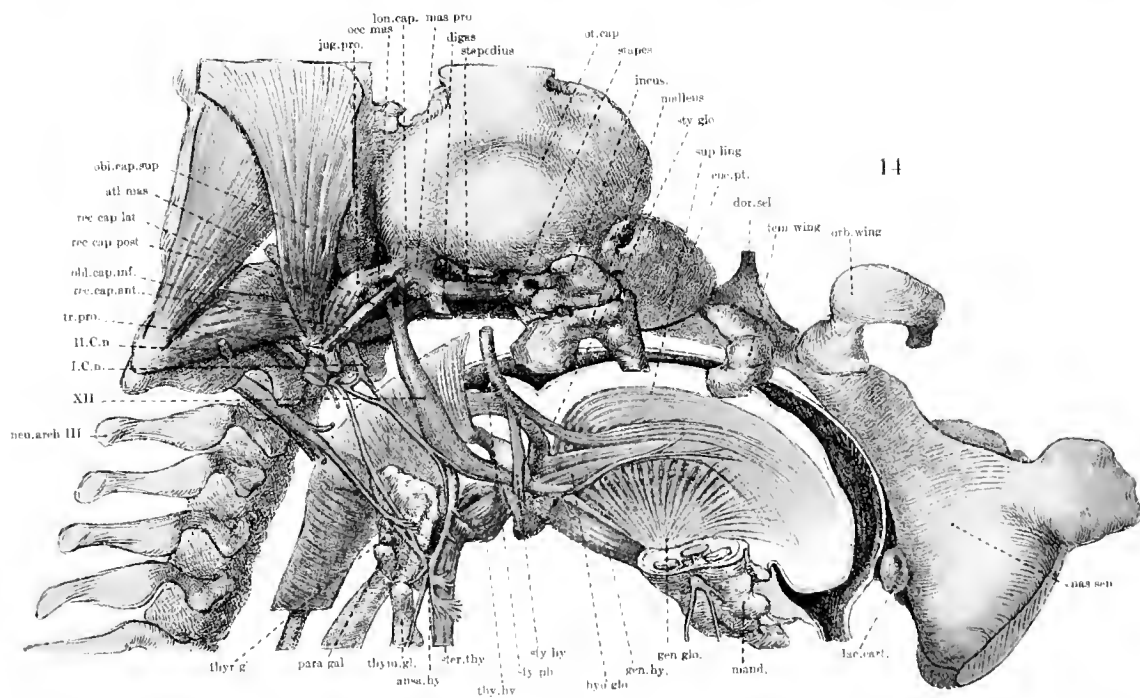
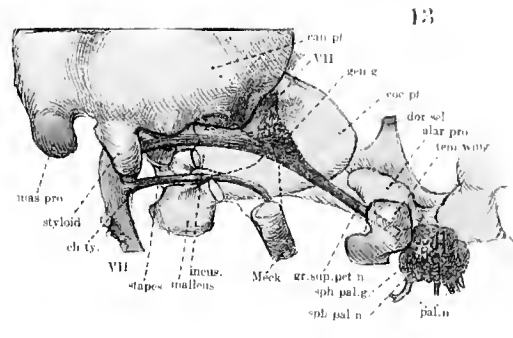
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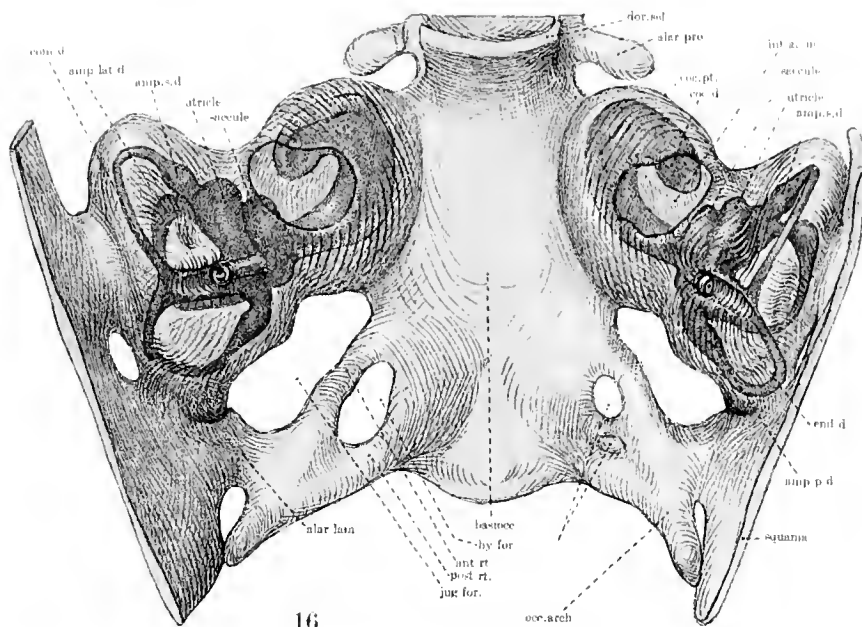
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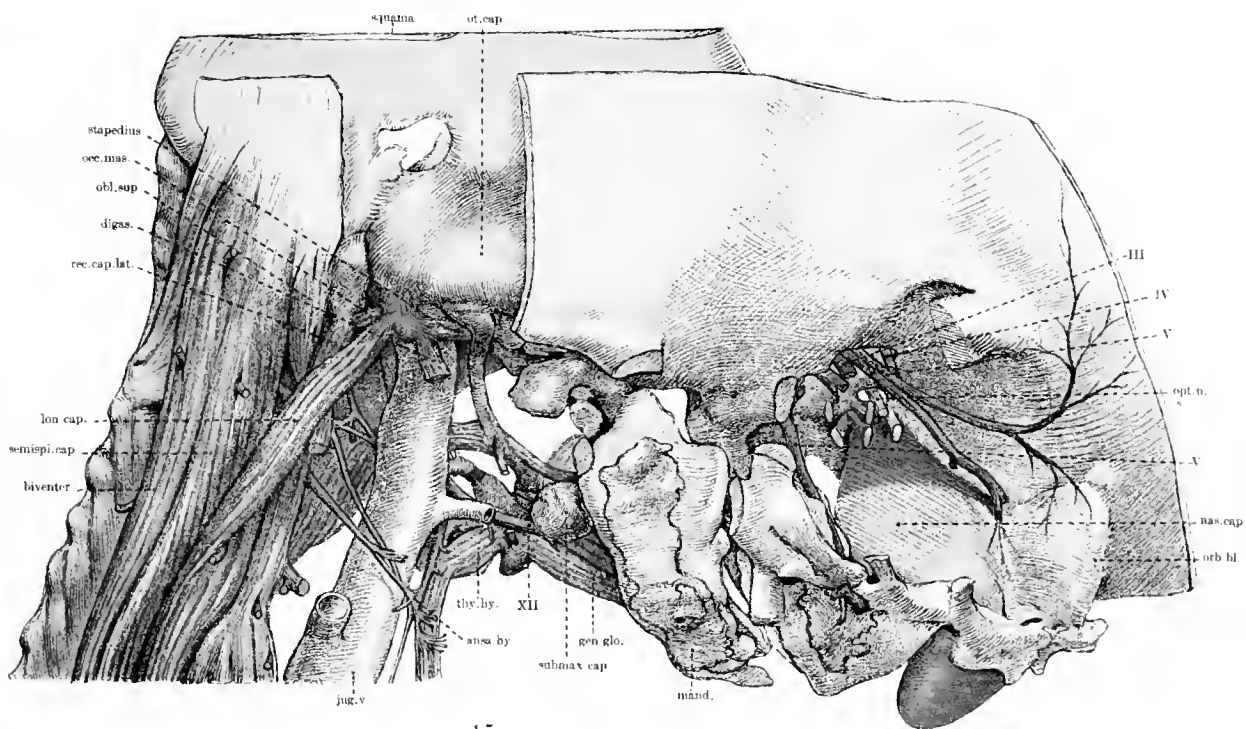
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HYDATIFORM DEGENERATION IN TUBAL AND  
UTERINE PREGNANCY.

BY ARTHUR WILLIAM MEYER,  
*Professor of Anatomy in the Leland Stanford Jr. University.*

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With six plates.

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# HYDATIFORM DEGENERATION IN TUBAL AND UTERINE PREGNANCY.

BY ARTHUR WILLIAM MEYER.

The following study is an outgrowth of a survey (planned by Mall) of the embryological collection of the Carnegie Institution of Washington. It was my privilege to share in this undertaking and to be permitted to follow any matters of special interest to me. The following report concerns itself especially with the occurrence of hydatiform degeneration in abortuses and specimens in the Mall Collection which were obtained through operation and were classed as pathological. My attention was attracted to the subject while engaged in an examination of the Hofbauer cells, begun at the suggestion of Mall. For the purpose of convenience I shall discuss the tubal and uterine cases separately, including what is common to both with the latter.

## TUBAL.

Strangely enough, the occurrence of chorio-epithelioma arising from tuba pregnancy seems to be better known and also better established than the occurrence of hydatiform mole within the tube. This is especially surprising in view of the stress laid by Marchand (1898) upon epithelial proliferation in cases of hydatiform mole and in view of the fact that trophoblast formation and epithelial proliferation in general have been regarded as being greater in tubal than in cases of uterine implantation. This is illustrated well by such cases as that of Fellner (1903), in which it was impossible to distinguish by histologic examination between the epithelial proliferation present in a case of tubal pregnancy and that from a chorio-epithelioma. From these circumstances alone it seems to me that one might expect hydatiform degeneration to be relatively more common in the tubes. Moreover, when it is recalled that experts still regard it as impossible to decide upon the question of malignancy or benignity in cases of suspected uterine chorio-epithelioma from histologic preparations alone, this surmise gains more in probability. The presence of hyperactivity in the trophoblast in many cases of tubal pregnancy as compared with the uterine was confirmed also by personal observation, and if, as stated by Teacher (1903), chorio-epithelioma arose in hydatiform moles in approximately 40 per cent of 287 cases, and according to Seitz (1904) and Fraenkel (1910) even in 50 per cent, the occurrence of hydatiform degeneration in tubal pregnancy can hardly be doubted because of this fact alone. Nevertheless, of the 7 cases of tubal hydatiform moles cited by him, Werth (1904) regards only the case reported separately by von Recklinghausen (1889) and by Freund (1889) as well authenticated. Werth reserves judgment, however, on the case of Matwejew and Sykow (1901), a report upon which was accessible to him, and to me, in a short review only. Seitz, however, accepted the short review of this case as convincing, nor did he ques-

tion the case of Otto (1871), or that of Wenzel (1893), and he incorrectly credited Wenzel with two cases. Werth, on the contrary, regarded these last two cases, and also that of Croom (1895), which is accepted also by Veit (1899), as undoubted instances of "simple hydropic degeneration of the connective tissue of the villi so common in aborted chorionic vesicles, both from the tubes and from the uterus." Werth unfortunately does not state just what he means by simple hydropic degeneration, but since he speaks of it as common in aborted ova, one may conclude that he refers to changes in the chorionic vesicle which have followed its isolation within the uterus after complete detachment from its implantation site. For want of a better term, such changes may, I presume, be spoken of as maceration changes, although usually they occur under non-putrefactive conditions. However, I do not thereby imply that these changes are similar under sterile and under putrefactive conditions.

Since Werth speaks of simple hydropic degeneration in aborted ova he does not, I take it, refer to a dropsical condition of the villi possibly due to an obstruction of the venous return, for such a condition necessarily would be rare and not common. Moreover, this condition of necessity would have to arise before and not after the death of the embryo and detachment of the chorionic vesicle. As in one of the cases of Hiess (1914), such a specimen also should contain blood-vessels—for, as emphasized also by Ballantyne (1913), the hydatiform villus is not merely an edematous villus.

That any one at all familiar with hydatiform degeneration, in its earlier as well as its later forms, upon gross and microscopic examination, could confuse it with maceration changes in a fairly well-preserved specimen in any but its very earliest stages does not seem possible to me. Normal villi contain capillaries, not to mention other things characteristic of them. Hydatiform villi, on the contrary, do not contain them, or only very rarely so, and in the early stages. When a villus becomes hydatiform—that is, when liquefaction of the stroma occurs—this liquefaction appears in more or less restricted portions of the villus, thus giving rise to the long fusiform and later spherical vesicles so characteristic of hydatiform mole. But when a villus becomes macerated the change is general, and usually also is noticeable in the embryonic and chorionic membrane itself, or at least within the epithelium. The latter usually is lifted from the stroma here and there, the caliber of the entire villus is increased, and the capillaries and the stroma show maceration changes as the villus becomes more translucent. This increase in caliber of the entire villus is not due to local liquefaction of the stroma, but to the pseudo-edema occurring in a villus of normal structure and form. In hydatiform moles, on the contrary, the epithelium not only is firmly attached but usually hyperactive. The changes characteristic of hydatiform degeneration may and often do appear in the villi while they still are implanted, and not only after the chorionic vesicles are detached. This does not imply, however, that the villi of a detached hydatiform mole can not also undergo maceration changes. They, of course, frequently do so, and it is in such cases as these that differentiation may be difficult or impossible, especially if it is to be made from an examination of ill-preserved fragments



only. The same thing is true also of the villi in the early stages of hydatiform degeneration and maceration, especially when the latter masks the former. The difficulty would be still greater in case of whole chorionic vesicles which are almost completely dissolved, leaving only a shadow picture formed by a coagulum without nuclei, which nevertheless may retain almost perfectly the form of the chorionic vesicle and of the individual villi. It may long be impossible to differentiate such cases as these, but they form only a relatively small proportion of the whole. The many cases both of uterine and tubal chorionic vesicles which still were implanted and show exceedingly fine instances of hydatiform degeneration, as well as the many splendid examples of groups of villi which were still implanted in the tube or in the decidua, and which were equally good examples of hydatiform degeneration, leave no room for doubt as to the frequency of occurrence of this condition, even after due allowance is made for the doubtful cases.

Werth further concluded that not one of the 7 cases of chorio-epithelioma regarded as having arisen from tubal pregnancies recorded before 1904 was sufficiently authenticated. Nevertheless, by 1910 Veit felt justified in saying that a considerable number of cases of chorio-epithelioma arising from tubal pregnancies had been described. He added that Risel (1895) gathered 11 cases from the literature and that the second case had been reported since Risel's paper. Since my interest in the subject is largely incidental, I have not taken the trouble to gather from the literature cases of chorio-epithelioma alleged to have arisen from tubal pregnancies which may have been reported since Veit wrote. Moreover, I could not presume to judge these cases critically. Hence I will accept the fact that chorio-epithelioma arising from tubal pregnancy is regarded as established by a number of investigators. If the conception regarding the relation of chorio-epithelioma to hydatiform mole is justified, then the occurrence of hydatiform degeneration in tubal pregnancy must follow on *a priori* grounds alone. Moreover, whatever the causes of hydatiform degeneration may be, one possibly is safe in assuming that the condition is not restricted to the uterus, and when I noticed that hydatiform degeneration was so very common in young uterine abortuses the surmise that it might be still more common in cases of tubal pregnancy seemed justified. Since over 100 specimens of tubal pregnancies from the Mall Collection were included in the survey originally planned by him, a study of these specimens formed an excellent opportunity for observations on this subject.

That the case of Otto, with its pathetic history, really was one of hydatiform mole, can not be doubted in view of the careful description of the whole case—its clinical history, necropsy, and the histologic examination. This case is interesting also because the degeneration was in its early stages, the hydatids being only as large as a pinhead and the embryo still being present. Moreover, from Otto's description it is very likely that the specimen contained Hofbauer cells which I have discussed elsewhere (Meyer, 1919).

The history of the case observed by Wenzel in 1855 and reported in 1893 is equally complete and equally pathetic, as could be surmised by all familiar with the history of tubal pregnancy. In this case the mole was as large as a "hen egg,"

the hydatids varied in size from a dot to a "bird cherry" (wild? cherry), and the degeneration was universal, although the menstrual age of this specimen was given as only 51 days. It is significant that Wenzel expresses surprise that even excellent handbooks of the day had nothing to say about hydatiform mole in cases of tubal pregnancy, except perhaps to refer to the case of Otto. Nor does the case of Wenzel seem to be the first one observed or that of Otto the first one reported, for Storch (1878), in truly epochal, though largely ignored, observations on hydatiform mole, cites Hennig (1876) as stating that two cases of moles in the tube were reported by Blasius (very likely E. Blasius, 1802-75). Since Storch wrote on hydatiform mole it is implied that Blasius saw one of these and not one of another type of mole, and since hydatiform mole is such a striking condition and has evoked much more interest than the other forms, an observation regarding it in the tubes well might travel down the decades, particularly since until recently the occurrence of hydatiform degeneration in the tubes was regarded as extremely rare. This is indicated also by the fact that Menu (1899) still referred to the case of Otto as a curiosity.

Pazzi (1908<sup>3</sup>) states that two cases of extrauterine moles have been described each by Hennig (1872), Farrell (1893), Donald (1902), and one case each by Otto, Freund, Theileher, Maret, Matwjew (Matwejew?) and Sycow (Sykow?), Bland Sutton, and one case of ovarian mole by Wenzel (1893). Wilkinson is said to have described a case of rupture of the tube with reduction of the mole to the size of a cherry, and Lob (1902) also gives a case of molar tubal pregnancy without cessation of menstruation. Since I am quoting Pazzi essentially verbatim, it is evident that he did not read the literature critically or discriminate between ordinary and hydatiform moles, but was misled by the old inclusive and confusing usage of the terms *mole* and *molar*, still current at the present day.

Krueger (1909) also reported a case of hydatiform mole with a cyst as large as a "walnut." The pedicle was 4 cm. long and attached to the amnion near the insertion of the cord. Krueger spoke of this as a placental cyst but regarded it as a hydatiform-mole-like structure which, microscopically, was limited to a single villus. If this were the only evidence presented by Krueger one might well question the nature of the cyst, but he added that microscopically the beginnings of hydatiform formations could be recognized on other villi also. Hence it would seem that Krueger's case must be added to the authenticated cases of hydatiform degeneration in the tubes.

So far as I am able to learn, then, the literature contains reports of 9 cases of hydatiform mole occurring in the tube, but two or three of these cases are not well authenticated. These 9 cases are formed by the 2 cases of Blasius or Hennig, that of Otto, of von Recklinghausen and Freund, and of Wenzel, the 2 of Croom, that of Matwejew and Sykow, and that of Krueger. A critical reading of Hennig's book on diseases of the tubes and tubal pregnancy makes it quite clear, however, that Hennig merely said that Blasius discovered "tubal moles" and that he observed two, and Behm one case of abortion of tubal moles. From the context also it is very clear that Hennig was not discussing hydatiform moles, although it is not

possible to say whether he meant that he himself or Blasius observed two cases. I should judge that the latter is the idea it was meant to convey. To these 7 authenticated cases I would add that of Maxwell (1910). In reading Maxwell's description one must feel that he himself regarded the case as one of hydatiform mole, but deferred to the opinion of the "Committee." This is suggested also by the title of his article. The illustration which accompanies Maxwell's article is so very suggestive, and his description so characteristic of hydatiform mole, that it seems very probable indeed that the specimen really was such. Maxwell states, for example, that "sections of the villi embedded in the wall of the tube have the typical structureless, bloated appearance of such pathological villi; and though there is no central cavitation in the villi, their structure, associated with the active proliferation of the Langhans layer, suggests that one is looking at a stage just short of vesicle formation." Moreover, as I am about to show, hydatiform mole is so very common both in tubal pregnancies and in uterine abortions as to increase still further the likelihood that Maxwell's case actually was one of hydatiform mole. This is merely an opinion, and only a complete description or an examination of the specimen itself could decide the matter.

In connection with what was said before, it is interesting that Maxwell also emphasized that epiblastic activity is increased in all abnormal sites of implantation, and any one interested in the problems of tubal pregnancy and acquainted with Mall's (1915) findings will be struck by Maxwell's statement that microscopical examination of many cases of tubal gestation lends no weight to the view that chronic inflammation of the tubes is at all a common causal factor of tubal pregnancy. Nor can I refrain, in this connection, from quoting the uncontradicted opinion of Doran, expressed in the discussion of Maxwell's case, that tubal gestation "probably represents some general deterioration in the generative power among civilized women."

To the 8 cases contained in the literature I wish to add 48 found among the first 1,187 accessions from the Mall Collection. Nor is it necessary to stop with these, for this collection contains many more not here included. It is merely a matter of recognizing the specimens by a routine examination, and since this paper has been written a number of specimens have been recognized among the daily accessions of tubes received through the unselfish efforts and the scientific interest of practitioners in all parts of the nation.

In addition to over 100 free specimens of uterine hydatiform degeneration, I have also seen more than a dozen fine specimens in large sections of uterine implantation sites, and some entire specimens still embedded in pregnant uteri and tubes. Indeed, how many cases of hydatiform degeneration one can find in conceptuses in tubal or hysterectomy specimens will depend very much upon the care with which the examination is made, for the condition undoubtedly is extremely *common*, and not *rare*, as heretofore supposed.

Although the alleged menstrual age of these conceptuses ranged approximately from 6 to 218 days, most of them were young *empty* chorionic vesicles or mere remnants of such. Portions of quite a number still were implanted within the tubes,

however, and among these were two unusually fine ones in a rare specimen of twin pregnancy in the tube donated by Dr. Cecil E. Vest, of Baltimore. Since the question of superfetation has been raised also in connection with twin tubal pregnancies, I hasten to add that such a phenomenon, even if it ever occurs (which seems exceedingly doubtful) can be excluded absolutely in this case. Both chorionic vesicles were approximately of the same size and lay in practically the same cross-section of the tube, the surfaces of contact being flattened.

Before proceeding with the statistical findings, I may say that the abortuses in the Mall Collection regarded as pathological are grouped (1) as villi only; (2) as empty or partial chorionic vesicles; (3) as chorionic vesicles containing some or all of the amnion; (4) all specimens containing nodular, or (5) cylindrical embryos, or (6) stunted, and (7) macerated and mummified fetuses. Any one interested in this classification will find it discussed and exemplified in an article by Mall (1917).

There were 40 tubes containing villi only, and in 14 of these hydatiform degeneration probably was present. In 10 specimens its presence was undoubted, but in 4 it was probable only. I realize that this margin of probability is exceedingly large, but this is easily understood if it is recalled that often only a few degenerate villi embedded in clot were contained in the cross-sections of many of the tubes, and that only a few sections were examined, not, of course, a complete series of each tube. Had the entire tubes been examined, or if more villi had been present, and if those present had been better preserved, the difficulty would have been almost wholly obviated. However, it is idle to set forth these things, because such conditions never will obtain, and the margin of probability becomes greatly reduced if it is remembered that in a large series the specimens necessarily supplement each other. Moreover, the changes in the villi often are so typical that they are unmistakable, even if only a few villi are present. Besides, examination in complete series undoubtedly would increase, not decrease the number found. In some of the doubtful cases the existence of hydatiform degeneration became probable only upon comparison with the many uterine specimens previously examined.

The evidence offered by the 36 tubal specimens in the second group, which is composed of empty chorionic vesicles or parts thereof, was very conclusive, for the cut portions of most of these tubes contained considerable portions or even sections of whole chorionic vesicles, sometimes quite free from clot. Some of them were implanted almost perfectly in the wall of the tube, and although many of them were folded extremely and collapsed more or less, small areas of several were nevertheless implanted undisturbed within the tube. The villi in some of these implanted specimens were so characteristic and the whole picture so exquisite, that the specimens rightly belong among the very finest instances of hydatiform degeneration found anywhere so far. This is true in particular of the case of twin pregnancy received from Dr. Vest. In this specimen the two chorionic vesicles, the intervillous spaces of which were devoid of blood, lay in almost the same transverse diameter of the tube and hence had distended the latter considerably. Both were implanted quite well over the entire area of contact, which included the whole perimeter of the tube. The chorionic vesicles were flattened at the region of mutual contact, which

divided the tube somewhat unequally, as shown in figure 1. Although the embryo and the amnion long had disintegrated completely, and although the chorionic membrane itself is thin, covered by degenerate epithelium and also disintegrating, the epithelium of the villi not only is well preserved but is accompanied by large masses of trophoblast and considerable syncytium. Syncytial buds are found on the chorionic membrane also. The tubal mucosa is largely and the tubal wall partly destroyed by the invading trophoblast. Only a few small vestiges of the walls of the villous vessels remain, and the stroma of all the villi has undergone changes characteristic of hydatiform degeneration represented in figure 2. One villus also contains an epithelial cyst resulting from epithelial invagination with subsequent isolation of the distal extremity, a process to be referred to later in connection with uterine specimens. Since most of the villi of this and similar specimens still are implanted in the tube, there can no longer be any question as to the time in which hydatiform changes in the stroma of the villi may be inaugurated. As illustrated in other instances in which isolated and small groups of villi still were implanted, the advent of degeneration of the stroma occurs, in part at least, before the villus is detached. Hence it is not merely a post-mortem or maceration change.

Another very interesting specimen of tubal implantation is No. 1771, received from Dr. H. M. N. Wynne, of the Johns Hopkins Hospital. The menstrual age of this specimen is 49 days, but its anatomic age, as based upon length according to Dr. Streeter's curve (unpublished), is 37 days, thus showing a discrepancy between the menstrual and anatomic ages of 12 days. The embryonic length is only 12.5 mm., although with a menstrual age of 49 days it should be at least 18 mm. Upon examination, Dr. Streeter found the chorionic vesicle to contain a good deal of magma, some of which still was adherent to the embryo, as figure 3 shows. As has been repeatedly emphasized in the literature, the presence of this coagulum in itself probably indicates that the embryo died some time previously.

The wall of the tube is quite thin, as figure 4 shows, but the implantation is fairly well preserved around the whole perimeter of the specimen. The mucosa is destroyed throughout the greater extent of the section and the trophoblast is abundant, except in one rather degenerate and hemorrhagic area. The chorionic membrane is thin but contains some vessels distended with blood. The stroma of many of the villi also contains vessels filled with blood, but the vessels in many others are very evidently in degeneration. The syncytium is scanty and many of the villi are very plainly hydatiform, as seen in figures 5 and 6.

A third exceptionally fine specimen of tubal hydatiform mole is No. 2052, donated by Dr. N. M. Davis, of Washington, D. C. Figure 7 shows a portion of the tube containing the hydatiform mole, some hydatiform villi of which protrude through an incision in the wall of the tube. The whole opening is filled with typical hydatiform villi barely detected by the unaided eye but perfectly evident under an enlargement of 4 diameters. They present an extremely fine picture when seen with the binocular under a magnification of 10 to 20 diameters. Examination under a higher magnification shows that the preservation of the specimen is unusually good and that all the villi are markedly hydatiform. Trophoblastic proliferation is so marked that in some places it gives the appearance of decidual formation.

Relatively little syneytium is present, but the trophoblast invades the muscularis in many places and a good deal of coagulum is present, most of it apparently having arisen from degeneration changes in the stroma of the mucosa and from similar changes in the trophoblast and the muscularis. The latter is moderately invaded by round cells. No remnant of the wall of the chorionic vesicle or of the amnion or embryo could be detected in the sections examined, both evidently having been absorbed completely, only some of the villi remaining behind; or, the chorionic vesicle may have been aborted and these villi left implanted within the tube.

Some exceedingly fine hydatiform villous trees were found among the specimens in this group. Scaffoldings or frameworks formed by proliferating syneytium arising from the epithelium of the chorionic membrane also were seen. Since the syneytial buds were found far out on proliferations of trophoblast which capped the villi, and also in the center of trophoblastic nodules, the origin of the syneytium from the Langhans layer would seem to be again and exceptionally well confirmed. In some cases a detached hydatiform villus was fastened by opposite extremities to two portions of the tube wall. It is well to remember, however, that one of these attachments probably was gained before the separation of the particular villus from the chorionic vesicle.

Of the 36 cases remaining in this group of chorionic vesicles without amnion, after deducting 8 (7 of which belong in group 1 and 1 in group 2), 50 per cent showed the presence of undoubted hydatiform degeneration and in 1 additional case its existence was doubtful.

Since only a few specimens are contained in each of the last five groups, I shall treat them as one. Among 28 specimens remaining in these groups 12, or 43 per cent, showed the presence of hydatiform degeneration and 4 others were doubtful. From this percentage it is evident that the incidence of hydatiform degeneration among tubal specimens seems to increase with advancing age of the conceptus rather than decrease, as will be emphasized in connection with the uterine specimens to be considered later. This probably can be attributed to the fact that the specimens in the first group are composed of villi only, and that many of the empty chorionic vesicles in group 2 were detached from the wall of the tube by hemorrhage before hydatiform degeneration had developed sufficiently to enable me to recognize it. Moreover, it must be remembered that all tubal specimens, no matter in what group they are classified, are in fact young specimens, and since those falling in the latter groups succeeded in maintaining a foothold in spite of repeated hemorrhages, a larger number of them might be expected rightly to show the presence of a hydatiform change.

The incidence of hydatiform degeneration in the 104 tubal pregnancies classed as pathologic is 44, or 42.3 per cent of the whole. This is a somewhat higher incidence than was obtained in the 348 uterine abortuses classed as pathologic, and may be accounted for partly, or wholly even, by the greater incidence of young specimens in the tubal series. That the tubal specimens undoubtedly were younger follows from common knowledge regarding tubal pregnancies alone, but it also is shown by the average menstrual ages, which were 43.4 days in 25 tubal, as compared

with 66.6 days in 51 uterine specimens. Moreover, 32 of the 48 tubal specimens of hydatiform degeneration, or 66.6 per cent, fall into the first two groups, thus again showing that the majority are small, young specimens.

Although the incidence of hydatiform degeneration among the pathologic tubal specimens is but slightly higher than that among the pathologic uterine specimens, the incidence of hydatiform degeneration in all tubal specimens contained among both the normal and pathologic is twice as high as that among the same classes of uterine specimens. This can be explained only partly by the fact that a larger proportion of the tubal specimens are young and pathologic. The pathologic tubal specimens form 69.2 per cent of 153 normal and pathologic tubal specimens found among the first 1,187 accessions, but the pathologic uterine specimens form only 33.6 per cent of the normal and pathologic uterine groups among the same accessions. But the real question remains, for the incidence of hydatiform degeneration among the specimens classed as pathologic was essentially the same in tube and uterus. Hence an increased incidence of 100 per cent in hydatiform degeneration in the tubes may be due to the less favorable nidus found there. If so, it throws a very significant light upon the probable cause of hydatiform degeneration, which would seem to lie in the conditions surrounding the implantation and early development rather than in the ova or spermatozoa themselves.

The conclusion reached in a study of uterine specimens that hydatiform degeneration is *absolutely less*, not *more* frequent near the menopause, is confirmed also by the study of the tubal specimens. The average age of 20 women in the tubal series was 33.9 years, as opposed to an average of 31 years obtained from 36 women in the uterine group. This age difference offers a tempting opportunity for generalization, and did the statistics include thousands of cases one might be willing to say that it points to a progressive change as cause, which begins in the uterus and finally reaches the tubes. But strangely enough, the average number of years of married life of 15 women in the tubal series is exactly the same as that of 29 women in the uterine series, or 7.1 years. This fact at once guards against a venturesome hypothesis, for it allows no longer period for the supposed ascending change to reach the tubes than the uterus.

Eight of 20 women from the tubal series had borne one child, 4 had borne two, and 3 more than two; thus again more than confirming the statistical findings in the uterine series, which show that 9 of 33 women had borne once and 18 but twice. The parallelism between these statistics is striking indeed, especially if the small numbers be considered; 14 of 23 women, or 60.8 per cent, in the tubal series had aborted but once, as compared to 19 out of 44, or 46.3 per cent in the uterine series, a fact which again points to the middle rather than to the end of the reproductive life of these women.

I do not know whether or not hydatiform degeneration in the tube also is *relatively* more common near the menopause, as will be shown to be the case in the uterus, for I have not been able to obtain data on the relative frequency of tubal pregnancy in the different decades in the reproductive life of women. However, since by far the greater number of pregnancies usually occur early in this period, it



probably would be safe to assume that most of the tubal pregnancies occur also at this time. Consequently, it might well follow that the ratio of tubal hydatiform degeneration to the number of pregnancies occurring in the later actually might be greater than that in the *earlier* decades.

The structural changes in hydatiform degeneration will be considered more fully in connection with the uterine cases. Suffice it to say that since I directed my attention especially to hydatiform degeneration I have been able to recognize its presence repeatedly at sight in relatively young vesicles (1 cm. large) not only from uterine but also from tubal pregnancies. This is, of course, especially true in the former, for the chorionic vesicles of these often are quite characteristic, and if inspection with the unaided eye or with a reading glass under a magnification of 2 diameters fails to reveal the true nature of the specimen, examination with a binocular under a magnification of 10 or 20 diameters often makes immediate identification possible.

#### UTERINE.

To read the titles of articles on "molar" pregnancies which have appeared during the last few decades, even, is a rather wearisome task. By far the great majority of the articles concern themselves merely with the report of "a case" or (rarely) of "several cases" of hydatiform moles. The recent cancer literature stands in marked contrast to this, for not even the general practitioner would think of reporting a routine case of cancer of the breast, let us say. The significance of these facts is self-evident, and whatever else they may mean they do imply that hydatiform mole still is regarded as a *rare* condition. Indeed, many of those reporting "a case" frankly say so, and although the incidence of hydatiform degeneration is estimated variously by different authors and investigators, there seems to be entire agreement that it is a rare, even if not an extremely rare condition. This opinion seems to be shared even by those general practitioners whose long practice runs high up into the hundreds or even into the thousands of obstetrical cases. Indeed, many general practitioners declare that they have not seen a single case of hydatiform mole during the practice of a long life.

This prevailing opinion can not be attributed solely to the influence of the schools or to books, but is based upon the actual experience of the individual practitioner and upon his conception of what constitutes hydatiform degeneration. This is illustrated, for example, by Menu, who said that a small hydatiform mole weighs 300 grams, a large one 8,000, with an average weight in his series of cases of 1,700 grams. But even specialists in charge of hospitals have reported experiences similar to that of the general practitioner. Pazzi (1909), for example, stated that although he had observed more than 6,000 cases of labor in his private and hospital practice, he never met with a case of hydatiform mole. Moreover, it would seem that only some specialists have come to regard the condition as somewhat less rare than was heretofore supposed. This is well expressed by Williams (1917), who wrote: "Hydatiform mole is a rare disease, occurring, according to Madam Boivin, once in 20,000 cases. On the other hand, the statistics of Williamson would indicate



that it may be found but once in 2,400 cases." Williams adds, however, that in his own experience it occurred even more frequently than stated by Williamson; and Essen-Möller (1912), on the basis of 6,000 cases treated between 1899-1908, gives the incidence at the Frauen-Klinik at Lund as 3 per 1,000. My former colleague, De Lee (1915), in commenting on hydatiform degeneration, also stated that he has "frequently found in aborted ova one or more villi degenerate and forming vesicles"; and similar remarks were made also by others, notably by Müller (1847), Marchand (1895), Veit (1899), van der Hoeven (1900), Hiess (and according to him also by von Hecker), Langhans, Weber, and Fränkel. Findlay (1917) also regards "it as fair to conclude with Veit, Freund, and Dunger that abortive types of hydatiform mole are commonly overlooked," and although he gave no evidence for his opinion Weber (1892) insisted that hydatiform mole "occurs much oftener than we are led to believe from books or other literature." Essen-Möller says König gave an incidence of 1 per 728 cases. Pazzi (1908<sup>3</sup>) stated that Dubisay and Jennin found in 1903 that hydatiform degeneration occurs once in 2,000 pregnancies, and that Cortiguera in 1906 declared that the frequency of hydatiform mole has a discouraging variation of from 1 in 3,000 to 1 in 700 labors, but that in his personal experience Cortiguera saw one case in every 300 labors. The latter incidence is only slightly higher than that given by Essen-Möller for the clinic at Lund, and somewhat below that of Kroemer (1907), who found 15 hydatiform moles in 3,856 births, or one in every 257 cases. Mayer (1911) reported 10 instances among 3,105 cases of labor, an incidence of 1 in 310 cases, and it is only necessary to add that Donskoj (1911) stated that the incidence of hydatiform mole in 28,406 cases at the Frauenklinik at München, between the years 1884 and 1910, was only 1 for every 4,058 births, to emphasize the discouraging variation of which Cortiguera spoke. Donskoj also stated that Engel gave the incidence as 1 in 800, and Korn as 1 in 1,250 births. Such a surprising fluctuation in the apparent incidence in adjacent communities points to differences in conception of what constitutes a hydatiform mole, and also to differences in character of the material upon which the calculations are based.

The existence of hydatiform degeneration in far greater frequency than commonly supposed is indicated also by the records of the Department of Embryology of the Carnegie Institution of Washington. However, the material covered by these records is not identical with that upon which the above opinions, or those of other obstetricians are based. The opinion of the obstetrician is based upon material belonging very largely in the later months of pregnancy, while that in the Mall Collection, on the other hand, belongs very largely in the earlier months. Hence this material is not truly representative of the entire period of gestation, but the same thing is true of the material upon which the general practitioner, the obstetrician, and the gynecologists have based *their* opinions, for these are based largely upon material from the last months of pregnancy. Hence mainly the cases of hydatiform degeneration which survive come to their attention.

But unless we can assume that the incidence of hydatiform degeneration is constant during the whole period of gestation, its incidence at any particular time

of this period may very incorrectly express that at any other time. This could fail to be true only if the incidence of death of the conceptuses and their susceptibility to hydatiform degeneration were exactly uniform throughout every period of intrauterine life. But we know that neither is true, for it is common knowledge that by far the great majority of the cases of uterine hydatiform degeneration, recorded in the literature, are mature specimens of total or partial degeneration obtained in the later months of pregnancy. Although such specimens may contain villi in various stages of degeneration, they nevertheless represent end or near-end results. Like the fetuses which rarely accompany them, they are full-term or near-term products when regarded as hydatiform degenerations, and unless we are to assume that conceptuses once affected by hydatiform degeneration always survive up to this period, statistical deductions based upon the cases that do survive can give us little idea of the actual frequency of the condition throughout the entire period of antenatal life.

That the specimens upon which past and also present opinion is based usually were large, is confirmed by the belief in the prevailing clinical criterion of the existence of a disproportionately large uterus in cases of hydatiform mole. The emphasis laid on this by clinicians is well illustrated by Seitz, who says that cases in which the uterus is too small are the exception. Indeed, it seems that the validity of this clinical dictum has been questioned only very recently by Briggs (1912). Since most early conceptuses showing hydatiform degeneration have been inhibited in growth before being aborted, it probably is only the specimens which persist that produce a uterine enlargement greater than could normally be expected. However, since—as emphasized by Gierse (1847), Storeh, Hiess, and others—most hydatiform moles are expelled early and spontaneously, it is evident that these can not have been adherent—that is, have penetrated very deeply—or they would not have been expelled early and spontaneously. Furthermore, maceration changes so commonly present in aborted hydatiform moles indicate very clearly that a large percentage of them, together with the decidua, had been more or less completely detached from the uterine wall some time before abortion occurred.

As far as one can gather from the literature, the present opinion regarding the incidence of hydatiform degeneration would be paralleled quite correctly if, in the case of measles, we assumed that it was as common in octogenarians as in children. Measles, indeed, is an extremely rare disease in advanced age, but it nevertheless is very common in infancy. This is exactly the mistake we have made regarding hydatiform degeneration. It may be and undoubtedly is a rare disease at or near term, as Gierse also stated, but it probably is the commonest of all diseases during the earliest months of gestation. The typical large hydatiform mole is an end result which it has taken long months to develop. No one seems to have followed its evolution, although hydatiform degeneration, whether total or partial, is, of course, gradual in its advent.

The records of the Mall Collection contained 8 cases of hydatiform mole in the first 2,400 accessions, showing a frequency 8 times as great as that given by Williamson, or an excess of 700 per cent. Since the first 2,400 accessions contain

309 cases of tubal and also 2 of ovarian pregnancy, only 2,089 uterine specimens remain. Hence the recorded incidence in the uterine specimens really is 8 in 2,089, or 1 in every 261 cases. This incidence is only slightly lower than that of Kroemer, and somewhat higher per 1,000 than that given by Essen-Möller for the Frauenklinik at Lund, or the personal experience of Cortiguera.

The highest incidence of hydatiform degeneration previously reported is that of Storch, who estimated it as 50 per cent, but he unfortunately did not give a record of his cases. However, Storch emphasized that the typical complete hydatiform mole is a relatively rare form of the disease, and that all manner of transition forms between the normal chorionic vesicle and the completely degenerated one can be shown to exist. Storch further emphasized the commonness of hydatiform degeneration, especially in the early months of pregnancy, but as Veit (1899) well said, Storch somehow has not received sufficient credit for his investigations. Gierse was forgotten completely. This seems strange, especially in view of the fact that Storch's work was done in Copenhagen, where Panum (1860) had done and still was doing such fine and very suggestive, indeed epochal, work on the origin of monsters. Although Storch devoted part of his paper to myxoma fibrosum, and reported only 5 cases of hydatiform mole, one of which, however, accompanied a living fetus, his opinions on the whole were far ahead of his time. In order to make this clear I shall quote a very significant passage, which indeed needs but slight changes to serve as a conclusion for my own investigations:

“Nun sind aber bekanntlich Eier mit blasiger Degeneration der Zotten und fehlerhaft oder nicht entwickeltem Fötus ein sehr häufiger Befund bei Aborten aus den ersten Schwangerschaftsmonaten. Mehrere solche Eier sind schon in den bekannten Arbeiten von Dohrn und Hegar beschrieben worden. Ich habe im Laufe des letzten Jahres eine grössere Anzahl von Aborten untersucht und derartige kranke Eier in mehr als der Hälfte der Fälle gefunden. Nicht selten ist die Amnionblase völlig leer und enthält nur eine klare seröse Flüssigkeit. In anderen Fällen sitzt an der einen oder anderen Stelle der Innenfläche des Amnion ein kleiner rundlicher oder unregelmässig geformter,  $\frac{1}{2}$ –1 Mm. grosser Körper, welcher aus Nichts als aus runden, schwach conturirten, zum Theil fettig entarteten Zellen und einer hellen, fast homogenen Zwischensubstanz besteht, und der durch einen feinen, 1–3 Mm. langen Strang von ähnlicher Natur mit dem Amnion verbunden ist. In noch anderen Eiern ist der Embryo zwar etwas weiter entwickelt, aber von den verschiedensten Formen von Missbildungen befallen. Seltener ist der Embryo einigermaassen wohl gebildet und von bis zu 2 Cm. Länge, wie dies auch Hohl nur einmal gefunden hat. Sehr gewöhnlich ist fettige oder lipoide Entartung des Embryo vorhanden; derselbe ist dann eine kürzere oder längere Zeit vor der Geburt abgestorben. Als die äussersten Glieder dieser Reihe von kranken Eiern stehen endlich die sehr seltenen Fälle, in welchen der Embryo seine Entwicklung ziemlich ungestört fortgesetzt zu haben scheint, und von denen die Fälle von Martin und der oben beschriebene dreimonatliche abort Beispiele sind.

“Die blasige Entartung der Chorionzotten kann demnach neben den verschiedensten Zuständen des Embryo gefunden werden. Sehr häufig ist letzterer der Sitz von mehr oder weniger eingreifenden Krankheitsprozessen gewesen, die in demselben verschiedene Missbildungen hervorgerufen und ihn in seiner Entwicklung gehemmt haben. Es sind diese Krankheitsprozesse wahrscheinlich immer sehr früh im Ei entstanden, und müssen mit Panum zunächst als entzündliche Vorgänge aufgefasst werden, welche nach ihrer Intensität und vielleicht nach dem Zeitpunkte, zu welchem sie im Keime auftreten, bald eine theil-

weise Verödung der Keimanlagen der meisten wichtigeren Organe mit Verkrüppelung des ganzen embryonalen Körpers, bald mehr locale Missbildungen einzelner Körperteile hervorrufen können. Das Erstere ist in den hier besprochenen Aborten sehr häufig der Fall; der Embryo ist zu einem unförmlichen Klumpen umgewandelt, dem die meisten Organe deren Keime durch Entzündung zerstört worden sind, gänzlich fehlen. Von diesen verkrüppelten Amorphi finden sich in anderen Eiern alle Uebergangsformen zu mehr oder weniger entwickelten Missbildungen was auch Panum an einigen Beispielen nachgewiesen hat. Es scheinen in der That die nicht zerstörten Keimzellen der verschiedenen Organe, nach dem ablaufe des Krankheitsprozesses, ihren ursprünglichen Entwicklungsplan mit einer oft merkwürdigen Hartnäckigkeit, so gut sie es können, festzuhalten. Von diesem Verhältnisse liefern die bekannten herzlosen Amorphi, die durch einen Zwilling Bruder ernährt werden und dadurch zu einer oft bedeutenden Grosse heranwachsen können, ein schlagendes Beispiel. In unseren Aborten sind zwar diese Amorphi, die keinen Zwilling Bruder zur Erhaltung ihres Kreislaufes gehabt haben, frühzeitig zu Grunde gegangen, und ihre Gewebsteile sind einer fettigen (lipoiden) Entartung anheimgefallen; sie haben jedoch ihre Entwicklung eine Zeit lang fortgesetzt.

“Est is von den verschiedenen Verfassern vielfach von einer Auflösung der Embryonen in der Amnionflüssigkeit und von einer nachherigen Resorption derselben gesprochen worden. Ich glaube indessen, dass diesen Vorgängen eine sehr geringe Rolle beizulegen ist. Man findet in der That gewöhnlich Nichts, was auf eine solche Resorption deuten könne. Es scheinen vielmehr die abgestorbenen Embryonen auch lange nach ihrem Tode eine grosse Widerstandsfähigkeit gegen die Einwirkung, von Amnionflüssigkeit beizubehalten. Ich habe mehrmals ganz kleine, verkrüppelte Embryonen zwar fetig entartet, in ihrer Form aber völlig wohl erhalten, in Eiern gefunden, die bis zu 10 Monaten im Uterus zurückgehalten worden sind. Zudem ist die Amnionflüssigkeit in diesen Eiern meist ganz klar, oder sie enthält nur losgestossene, hinfällige Amnionepithelzellen suspendirt. Wenn daher die Eier ganz leer gefunden werden, so rührt dies gewiss am Häufigsten daher, dass der Primitivstreifen seiner Zeit völlig destruiert worden und somit gar kein Embryo zur Entwicklung geworden ist. \* \* \* Im Allgemeinen erreichen sie keine bedeutende Grösse und werden zudem oft frühzeitig aus dem Uterus ausgestossen, in dem sie, wie oben besprochen, ein sehr beträchtliches Contingent zu den Aborten überhaupt liefern. \* \* \*

“Die Traubenmole und die verschiedenen Uebergangsformen derselben, die an Aborten sehr häufig vorgefunden werden, ist als Hyperplasie und secundäre cystoide Entartung des (von Allantois nicht herstammenden) Chorionbindegewebs vorzugsweise characterisirt. Die Krankheit wird von pathologischen Zuständen der übrigen Eitheile, Amnion und Embryo (Missbildungen, Verkrüppelungen und frühzeitigem Absterben des letzteren) sehr häufig begleitet. Seltener ist der Embryo regelamässig entwickelt, stirbt aber meist auch dann wegen mangelhafter Vascularisation der (Chorion) Placenta frühzeitig ab. Sehr selten scheint der Embryo ungestört bis zur Geburt sich fortentwickelt zu haben.”

But the unregarded observations and illustrations of Gierse are still more startling than these opinions and observations by Storeh, who knew of Gierse's observations published posthumously by Meekel. The latter quite correctly stated that such careful observations as those made by Gierse always introduce new points of view. If it be remembered that in these days, almost a century later, specimens of hydatiform degeneration which are 4 cm. in diameter still are reported separately as examples of *early* hydatiform degeneration, the great merit of Gierse's observations in this regard alone will be clearly evident, upon recalling that Gierse pictured a hydatiform villus from a chorionic vesicle the size of a hazelnut (about 12 mm.), the largest hydatids on which were only one-third of a line large. Moreover, Gierse added:

"Derleichen geringe krankhafte Veränderungen finden sich an anserordentlichen vielen Abortus, und sie scheinen die häufigste Ursache des Abortus in den ersten Monaten zu sein."

How such an epoch-making conclusion not only could be forgotten, but absolutely overlooked or disregarded, by all but a few of the scores upon scores who have written on hydatiform degeneration, it is difficult indeed to understand. Gierse, who took steps to ascertain what normal villi look like, stated that villi with marked irregularities as described by Desormaux, Breschet, Raspail, and Seiler undoubtedly were abnormal; surmised that villi in abortuses seldom are normal, and added that between the slight pathologic changes in the caliber of the villi and the most evident hydatiform moles the plainest transition can be found. Among other important things Gierse also recognized the early fenestration of the stroma and pictured such a villus under a magnification of 250 diameters, and although reported very briefly, his findings, wholly confirmed here, still wait for general recognition.

Just as the great majority of specimens described in the literature are large, so 4 of the 8 specimens originally classed as such in the Mall Collection also are large, and none of the 8 are very young, as the following protocols show:

No. 70 (Dr. Charles H. Ellis) is a small, firm, degenerate-looking, almost solid mass  $40 \times 30 \times 28$  mm., composed of small cysts, degenerate decidua, exudate and degeneration products. As figure 8 shows, it is very similar to a very much larger specimen, No. 323 (Dr. V. Van Williams). The latter is a large, firm, felt-like mass  $120 \times 90 \times 65$  mm., represented in figure 9. The individual cysts, which vary from 1 to 20 mm., are packed together rather firmly, though a few large ones are free. The exterior of the specimen is formed by a thick layer of degenerate decidua and gives only a slight indication of its true nature upon closer inspection or upon examination of the cut surface. No fetal remnants were noticed, and microscopic examination shows that the specimen is composed merely of a large hydatiform mass which was retained for a long time and then aborted *in toto* with the surrounding decidua and exudate.

No. 749 (Dr. G. C. McCormick), on the contrary, is a fresh, loose, typical hydatiform mass composed of loose hydatids of various sizes, as shown in figure 10. As the specimen floats loosely in fluid, it fills a half-liter jar about two-thirds. A considerable portion of the hydatid cysts are glued into a solid mass by blood, exudate, and decidua, which form a layer on the exterior.

No. 1323 (Dr. J. W. Schlieder) also is a large mass very like the preceding, which completely fills a liter jar. It is accompanied by much clot and composed mainly of a large, thick-walled, hemorrhagic, necrotic mass  $80 \times 50 \times 45$  mm., containing a large, thin-walled cavity  $65 \times 30 \times 25$  mm., which is broken at one end. This cavity, which is apparently that of the chorionic vesicle, is empty, smooth, and thin-walled, except where it is composed of a characteristic hydatiform mass shown in figure 11.

No. 1325 (Dr. Fred R. Ford), shown in figure 12, is a small, irregular mass  $40 \times 33 \times 20$  mm., the exterior of most of which is formed by a thin layer of decidua. Within this is a small group of quite typical hydatid cysts, the largest of which measures about  $10 \times 5$  mm. The appearance of the specimen suggests that it is merely a fragment, though the amount of decidua present indicates that the entire specimen probably was not much larger. The history of this specimen is especially interesting because of the diagnosis of tubal pregnancy, caused by the presence of a cornual myoma and the occurrence of repeated bleeding.

By far the most interesting specimen, in some respects, of hydatiform degeneration among those diagnosed as such upon gross examination in the Mall Collection is No. 1640.

This abortus, received through the courtesy of Dr. J. W. Williams, measured  $40 \times 20 \times 15$  mm. Upon examination Dr. G. L. Streeter found it to be composed of a flattened decidua and chorionic mass which, upon section, showed "pearl-like vesicular enlargements which suggest hydatiform degeneration." The exterior of this specimen is composed of a thin, hemorrhagic decidua which completely surrounds the villi. The hydatid nature of this clearly is recognizable upon close scrutiny with the unaided eye, and easily becomes evident upon magnification of 12 diameters with the binocular microscope. Examination of the histologic preparations reveals it to be a very fine specimen of relatively early hydatiform degeneration.

No. 1914 (Dr. G. C. McCormick) is a fine, very characteristic mass, part of which is shown in figure 13. It is like Nos. 749 and 1323, but very much larger, for in fluid it completely fills a 2-liter jar. This specimen was said to have accompanied a living, 7-months fetus, having been expelled between the fetus and the placenta. Only a small amount of clot, and what seems to be a small portion of placenta and membranes, accompanied it. Since the placenta was not saved it is impossible to say whether the mass resulted from partial degeneration of the placenta belonging to the living child, or whether it represented a degenerate twin placenta, which is rather unlikely but not impossible, in view of the well-authenticated cases found in the literature. This specimen is of interest not only for the numerous large, clear cysts, one of which measures  $30 \times 25$  mm., which it contains, but because it accompanied the birth of a living child and because of the relative rareness of such a coincidence. In regard to the latter, Dr. McCormick added that in his experience of over 1,000 labors he had never before met this coincidence. The rareness of the specimen is emphasized still further by the statement of Professor Williams that such an instance has not been observed in a series of over 17,930 obstetrical cases treated by the department of obstetrics of the Johns Hopkins Medical School, as well as by the small series of such cases recorded in the literature.

No. 1926, a companion specimen to No. 1640, is composed of material from curettage received through the courtesy of Dr. Karl Wilson, of the department of obstetrics of the Johns Hopkins Medical School. It was removed from the same patient about a year later than specimen No. 1640. Upon gross examination the hydropic nature of some of the villi is plainly evident, as shown in figure 14, and upon microscopic examination the diagnosis of hydatiform degeneration could be confirmed, although the villi were extremely degenerate. The menstrual history of this case fortunately is known and is thoroughly reliable. The last menstruation occurred January 24 and curettage was done August 4. Bleeding occurred every two or three weeks during March and April and was repeated throughout May. Since the uterus, which had reached the symphysis, had not enlarged any for months, in view of the long duration of pregnancy the operation was performed. The major portion of the specimen is very small. The chorio-decidual portion was felt-like in consistency and extremely fibrous, due largely no doubt to the long retention. Most of the accompanying material looks like mucosa rather than decidua, although some of the larger pieces very evidently contained villi. Some of these were relatively thick and fibrous, and others were vesicular. All of the material was extremely fibrous, making it difficult to get a satisfactory teased preparation. Accompanying this material was a small body  $5 \times 7.5 \times 30$  mm., shown in figure 15. Both nodule and stalk contained some remnants of the embryo. Although the appearance of the stalk suggests the umbilical cord, it contains fragments of the body of the embryo, some of which evidently are composed of nerve tissue.

Microscopic examination of the pedunculated mass further shows it to be composed of degenerate remnants of organs, tissues, and cells. It is partly denuded and partly covered by a layer of fibrous connective tissue which contains local thickenings. In other areas this fibrous layer gives place to a single or more celled layer, or to polygonal epithelioid cells. The interior of this specimen is composed of a degenerate jumble including frag-

ments of the central nervous system, of the heart, liver, and cartilages. The entire body is chaotic in its structure, and small fragments of the nervous system are scattered throughout its entire extent. This would seem to indicate that the disruption of the tissues was mechanical. The material in which these remnants are contained is composed of coagulum, some mesenchyme, cellular detritus, blood and polymorphonuclear leucocytes, degenerated cells, which appear to have been phagocytic, but which are more likely fusion products or "symplasma" (as Bonnet called them). A few remnants of vessels are found only in the fragments of cartilage.

This short review of the gross appearance of the cases of hydatiform degeneration recognized by the unaided eye with the customary criteria, originally classed as such in the Mall Collection, shows that they vary decidedly in their gross, naked-eye characteristics, both as to size and appearance. No. 1640 scarcely is distinguishable as a case of hydatiform degeneration from gross appearances alone, unless one's attention is directed especially to the matter, but all the rest of the specimens, both small and large, not only are easily recognizable, but are so characteristic that they could not possibly be overlooked. As was indicated above, the incidence of these specimens of hydatiform degeneration among the first 2,400 accessions in the Mall Collection was 1 in every 261 abortuses, or more than 8 times the incidence given by Williamson, and 1.3 times that given by Essen-Möller. Although this incidence is so much higher, it does not necessarily contradict the statements of Williamson, for it represents the incidence of hydatiform degeneration in abortuses belonging very largely below 7 months. Nor does it tell the whole story for these months, for since the incidence of hydatiform degeneration given in the records of the Mall Collection is based upon determinations made essentially in the usual way—that is, by unaided inspection of the gross specimen alone—we must regard it also merely as an apparent, not as the *actual* incidence. For, as will appear later, the actual incidence can be revealed only by a careful gross and microscopic study of all specimens, both normal and pathologic. Such a study has not as yet been completed, but 348 uterine specimens classed as pathologic, and 105 pathologic tubal specimens, contained in the first 1,187 accessions, were carefully examined.

The actual number of cases of hydatiform degeneration found among the 348 uterine abortuses classed as pathologic was 112, or 32.4 per cent of the whole. The incidence of hydatiform degeneration in the pathologic tubal pregnancies was somewhat higher even—44 specimens of undoubted hydatiform degeneration in 105, or 41.9 per cent. Since nearly all the tubal specimens are young, while the uterine series contains many more relatively older ones, the effect of this fact upon the determined relative incidence of hydatiform degeneration among the pathologic tubal and uterine specimens must be borne in mind. For a reliable conclusion regarding the relative incidence in the uterine and tubal pregnancies it would be necessary to select a series from each, composed of specimens of approximately corresponding ages. What the incidence of hydatiform degeneration is among the uterine and tubal specimens classed as normal I do not know, but it undoubtedly is far below that in those classed as pathologic. It is well to remember, however,



that many, if not most of the instances of beginning degeneration very likely will be found among the specimens classed as normal. This is well illustrated by a hysterectomy specimen, No. 836.

If we assume that the incidence of hydatiform degeneration among the pathologic specimens in the rest of the Mall Collection is the same as that among those in the first 1,187 accessions, then we get over 314 estimated instances of hydatiform degeneration in pathologic tubal and uterine cases alone. Since I have found a number of chorionic vesicles accompanying embryos classed as normal which also show hydatiform degeneration, this number would be increased still further; but unfortunately too few of the specimens classed as normal were examined to justify an estimate. Yet these normal specimens form 60.4 per cent of the first 1,000 and 40.7 per cent of the first 2,500 accessions. This supposed increase, due to inclusion of specimens contained among the normal, would be offset somewhat, however, by the fact that the first 1,000 accessions contain a somewhat larger proportion of young conceptuses, each succeeding 1,000 probably becoming somewhat more representative of actual life conditions. The difference between the composition of the first 1,000 accessions and that of the 1,000 between 1,500 and 2,500 is not very great, however, for the former contains only an excess of 17.6 per cent of cases falling in the first five groups of the Mall classification, which groups are composed largely of specimens below an embryonic length of 20 mm. Then, the relative proportions of tubal and uterine specimens in the different thousands also must be taken into consideration. But in any case the estimated incidence of hydatiform degeneration in the Mall Collection, calculated without regard to those contained among specimens classed as normal, is 7.5 per cent, and the actual incidence hence probably is more than 1 in every 10 accessions. The incidence among the uterine specimens alone would be 10.9 per cent, and among the tubal alone 20.8 per cent. This difference of 100 per cent between the tubal and uterine specimens may have a probable significance in connection with the cause of hydatiform degeneration.

If, as alleged by various investigators, the great majority of abortions occur in the first 3 months, it is highly probable that many of these early conceptuses are lost and never come to the attention of any one, and that therefore the proportion of early specimens in this or any other collection is no doubt too small. Moreover, in quite a number of specimens of the first 1,000 accessions the chorionic vesicles were too degenerate for examination, and in others they were absent, but we have reason to believe that this is not true to the same extent in the material beyond the first 1,000 accessions. Then, too, since only a few relatively large sections from a single portion of the chorionic vesicles were examined, it is evident that some cases in which the degeneration may have been purely local probably were overlooked. Hence the actual incidence of hydatiform degeneration in this collection is probably not merely 8 times but 240 times as great as that given by Williamson (1900), and 33.3 times as great as that given by Essen-Möller.

Most persons will, I presume, be willing to regard an increase of 700 per cent above that of Williamson as possible, but one of 24,000 per cent above Williamson, or even 3,333 per cent above that of Essen-Möller as wholly out of the question.



Yet, strange as it may seem at first sight, this is not a random guess but an estimate based upon the actual incidence of hydatiform degeneration as determined by a careful gross and microscopic examination of mounted and unmounted material from over 400 abortuses. However, I lay no special emphasis on these percentages, and am using them merely to emphasize the great frequency of hydatiform degeneration. It matters little whether we shall ultimately determine an incidence of 10 or 5 per cent, but it does matter considerably whether we regard the frequency as 5 or 0.05 per cent, for this is a difference of 10,000 per cent.

In view of the prevailing opinion, I realize that these findings may seem incomprehensible and perhaps incredible, unless it is distinctly borne in mind that it is not stated that this incidence refers to the later months of pregnancy or to term. What the incidence in the later months of pregnancy may be I do not know, but I have called attention to an apparently well-founded belief that it is a relatively rare condition, the estimates ranging from 1 in 2,000 to 1 in 728 or 300 cases.

In regard to the incidence of hydatiform degeneration in uterine specimens, it should also be remembered that the *life*, in contrast to the *laboratory* incidence for the entire period of gestation is higher, not only because the chorionic vesicles were not included in many of the accessions and because others were too degenerate, but because I have not as yet been able to recognize the very earliest stages with entire certainty. Furthermore, many instances of hydatiform degeneration from the early months of pregnancy, especially the first and second, are inevitably lost. The increase due to these things would be offset somewhat, however, by the lower incidence of hydatiform degeneration in specimens from the last months of pregnancy, relatively few abortuses from these months being contained in the Mall Collection.

To what extent the material in this Collection is truly representative of actual life conditions is difficult, if not impossible, to determine. This question could be answered only if all the abortuses and material from abortions actually reached physicians, and if the latter sent all of them to the laboratory. My own impression so far is that the material representative of a sufficiently large community probably would have a somewhat lower incidence, notwithstanding the fact that many specimens not only of hydatiform degeneration, but of abortuses in general, especially from the first month of pregnancy, are lost. However, since the presence of hydatiform degeneration is especially common among early specimens, the inclusion of these might raise the incidence for the whole period of gestation more than the inclusion of all specimens (not excepting those of the last three months) would lower it. But the result obtained would represent the incidence of hydatiform degeneration in abortuses alone, and not that in all pregnancies. The latter could be obtained only by including all gestations which end normally. If we accept Pearson's (1897) estimate that approximately 40 per cent of all pregnancies end prematurely, then the incidence of hydatiform degeneration among abortuses would represent very nearly twice that in all pregnancies. Mall's estimate of 20 per cent prenatal mortality, on the other hand, would give us an incidence only one-fifth as great as that among abortuses. Hence, the actual life incidence of hydatiform

degeneration in all gestations would then be 1 in 10, as based upon Pearson's, and 1 in 25, as based upon Mall's estimated prenatal mortality. But even if, as estimated upon this basis, 4 or 10 per cent of all conceptions end in hydatiform degeneration, this does not necessarily contradict the current opinion regarding its rareness at or near term.

A careful examination with the binocular microscope of all specimens has shown that hydatiform degeneration as a rule is sufficiently general even in young vesicles, so that sections of a single portion about 10 mm. square, would enable one to make a fairly reliable diagnosis. Now and then, however, the process seems to be rather irregularly developed, especially in the larger specimens.

In order to determine accurately the question of distribution of hydatiform degeneration over various portions of the chorionic vesicle, it will be necessary to examine a series of sections of portions of the chorionic vesicle for each small specimen. This has not yet been done, but since the portions used for microscopic examination had been taken at random *without previous knowledge of the existence of hydatiform degeneration* in any but the 8 specimens above described, and since a series of 453 vesicles was examined, I can not believe that it can often be limited to any particular area on relatively young vesicles. In these it usually is universal even if not complete. It is of special interest in this connection that Muggia (1915), after reviewing the small list of cases of alleged hydatiform degeneration of the chorion laeve in connection with a study of a case of his own, came to the conclusion that these cases are not really degenerations of the chorion laeve, but merely partial degenerations of the placenta. Although I have given no thorough attention to the normal changes in the chorion laeve, I am quite certain that they are not the cause of confusion in the series of hydatiform degenerations from the Mall Collection. Cases in which whole chorionic vesicles exquisitely hydatiform in character were contained in the tubes, and a number of others which still were implanted within the uteri showed equally exquisite hydatiform changes around the whole perimeter. Such cases as these ultimately confirm the opinion that in young vesicles as a rule the condition is general except at its very inception. This is true particularly by the time the degeneration has reached a stage which can be considered at all typical in its gross development, as determined by careful examination of numerous specimens with the binocular.

It is especially interesting that, just as soon as typical hydatid elliptical villi, or portions of the same begin to appear, the condition can be recognized with some certainty under a magnification of 12 to 20 diameters with the binocular microscope. It often was surprising how relatively early stages could thus be detected and the diagnosis confirmed later by histologic examination. Indeed, celloidin blocks of tissue from which sections had been cut gave splendid testimony when examined in fluid with the binocular. One of the not very early stages contained *in utero* and represented in figure 16 could be recognized with the unaided eye; and when examined with the binocular, under a magnification of about 12 diameters, the picture was unusually fine and wholly unmistakable, as shown in figure 17.

That hydatiform degeneration is incomparably more common in the earlier than in the later months of pregnancy, thus justifying the comparison made with measles, is substantiated by statistics covering the material examined. From these it is evident that, excepting cases of large hydatiform masses originally classed as hydatiform degeneration from inspection of the gross specimens alone, practically all the specimens are relatively young. This is true especially of those from tubal pregnancies, and we may hence regard it as established that hydatiform degeneration is a change which is exceedingly common in the earlier months of pregnancy, just as measles is common in childhood, and that it becomes progressively less common as the end of pregnancy is approached, just as does measles as senility is approached. The obstetrician does not see most of the cases of hydatiform degeneration, for they merely are reported as miscarriages and the specimens often are destroyed or retained unrecognized by the general practitioner or the midwife. They often are aborted spontaneously and completely with the decidua and rarely are still contained in a closed decidual cast when they reach the laboratory.

The spontaneity of the abortion, especially in early cases, was emphasized also by Storeh in the above quotation. Cortiguera (1906) is reported by Pazzi (1908<sup>3</sup>) also to have declared that many moles disappear wholly without leaving a remnant, even if occurring repeatedly in the same woman, and Donskoj also stated that many of those aborted do not come to the attention of physicians because of their harmlessness. This, however, does not imply that those which persist and develop into large masses are equally harmless, and it must be remembered that it is upon these that the current opinion regarding the tendencies to malignancy of the hydatiform mole is based.

The conclusion regarding the greater incidence of hydatiform degeneration in the early months of pregnancy is conclusively confirmed by the occurrence of 32 of the 48 tubal specimens within the first two classes of the pathologic division of Mall, and 104 of the 112 uterine specimens in the first six classes of this division. Most of the specimens in these classes are composed of villi, of empty chorionic vesicles, or of vesicles with embryos most of which have a length of less than 20 to 30 mm. That hydatiform degeneration is more common in the early months of pregnancy is indicated also by the well-known reports of Kehrer (1894) on 50 cases, and of Dorland and Gerson (1896), who found that 63 per cent of 100 cases had aborted in the fourth and fifth months of pregnancy. According to Seitz, Hirtzman (1874) also found that 62.8 per cent of 35 cases had aborted between the third and sixth month. Only 4 per cent of Kehrer's 50 cases and only 3 per cent of the cases of Dorland and Gerson aborted at the tenth month. Donskoj stated that 7 of the 10 cases reported by him aborted in the fourth month and none after the sixth month. He stated further that 56 per cent of Bloch's 50 cases aborted before the sixth month, 44 per cent later than this, one being retained until the fourteenth month. The latter case is especially interesting because retention not only beyond term but after the death of the mole seems to be regarded as relatively rare. This, however, does not imply that retention beyond the period of growth of the hydatid mole does not occur, although Sternberg (1910), who also emphasized the great

rarity of this condition, erroneously stated that the German literature reveals only a single instance of missed abortion in case of hydatiform mole, *viz.*, that of Poten (1901). In this case a hydatiform mole of the size of a duck egg was said to have been aborted approximately one month beyond term. Hence growth must have ceased long before and the mole have remained *in utero* as a "harmless body." To this case of Poten, Sternberg adds a case in which a hydatiform mole of  $14 \times 9.6 \times 4.3$  mm. was aborted in the twelfth month after the cessation of menstruation. Although Sternberg included 4 cases from other countries among these missed-abortion moles, *viz.*, those of Sheil (undated), Ferguson (also undated), Colorni (1908), and Gaifani (1908), one can hardly doubt that more cases could be added. Since the case of Sheil was one of twin pregnancy in which one conceptus became hydatiform, it is not at all unlikely that some other cases among this rather small series of twin pregnancies accompanied by hydatiform degeneration may belong in this category.

Mayer also emphasized the fact that, although instances of retention of fetuses are very common, instances of retention of hydatiform mole are very rare, only a few cases having been recorded. Mayer refers to 2 cases by Kehler, 3 of Dorland and Gerson, and to 1 case of Lange, and reports 4 of his own. These 4 were found among 10 cases of hydatiform mole, an incidence of retention of 40 per cent. They are interesting, especially in connection with the observation of Briggs that, contrary to current belief, uterine enlargement often is not beyond the normal. Mayer says that this enlargement was too great in but 1 of the 4 cases, and that retention lasted as long as 4 to 5 months.

At least 3 of the cases of hydatiform mole originally recorded as such in the Mall Collection belong among retained specimens, as the illustrations alone suggest. But a fair percentage of detached chorionic vesicles included in the list of cases here reported undoubtedly also was retained after the cessation of growth, and it is for this reason that I further emphasize the fact that the uterine volume in a considerable percentage of these cases also, instead of having been too great for the duration of the pregnancy unquestionably was too small. This is well illustrated by the histories of specimens Nos. 70, 323, 1640 and 1926, and by the specimens themselves.

The average menstrual age of 51 of 112 uterine specimens of this series of hydatiform degeneration—in which the data were available—was 66.6 days, or  $2\frac{1}{4}$  months. As will be seen, this is a far lower average age than heretofore reported, a difference which explains itself from what has been said already. It is interesting that the average menstrual age of 5 of the 8 specimens in the Mall Collection originally classed as hydatiform degenerations is 168.2 days, or  $2\frac{1}{2}$  times as great, thus being in substantial agreement with the usual results. Three of these 5 are large specimens, the fourth measures  $40 \times 20 \times 15$  mm., and the other is composed of small fragments contained in material from curettage. From this alone it follows that the menstrual age is a very uncertain guide, especially as to the size of a hydatiform mole.

It seems superfluous to add anything to the good description of the gross appearance of the *typical* hydatiform mole currently reported in the literature. Such cases are so characteristic that even a novice can recognize them at sight. Yet if the findings reported here are reliable, or even approximately so, it nevertheless must be evident that, in the past, the great majority of specimens of true hydatiform mole have remained unrecognized merely because they did not happen to present the customary, well-known picture *to the unaided eye*. Small chorionic vesicles, such as No. 2077 shown in natural size in figure 18, which attract no attention upon cursory inspection may, and often do, present the most exquisite picture of hydatiform degeneration when seen under a magnification of 3 to 20 diameters, as illustrated in figure 19. This is true especially if the examination is made with the binocular microscope. Since I have adopted this method of examination it has been possible to recognize instances of decidedly general and typical hydatiform degeneration in chorionic vesicles less than 2 cm. in size, with later confirmation of the diagnosis by a histologic examination. However, I have not been able to recognize very early stages merely by examination of the gross specimens, for gross recognition is possible only when portions of at least some of the villi have become sufficiently elliptical or globular to attract attention. Histologic recognition is possible far earlier than this, as shown in figure 20.

The general appearance of the whole chorionic vesicle sometimes is an aid in gross identification, for the villi not infrequently are smooth, slightly branched, and unusually long, so that the vesicle looks shaggy, as illustrated in figure 21. The typical gross, *hydatid* or *watery*, translucent nature of the villi can not be relied upon in early stages, for normally shaped villi, which have undergone considerable lysis, may be almost transparent and also somewhat more than normally bulbous. However, save in the case of some specimens of tubal pregnancy, the swelling of the villi, due to maceration or to luetic changes, is quite different in character from that characteristic of hydatiform degeneration, and usually quite easily distinguishable from it. Judging from several specimens of villi which were macerated in distilled water during a period of weeks, post partum maceration never could cause confusion and the same thing undoubtedly is true of intra-uterine maceration.

Since numerous trophoblastic nodules are present also in other conditions, notably in retained placenta as found by Asehoff and others, I have not been able to regard their presence in unusual numbers, in some cases of hydatiform degeneration, as of crucial value, but the absence of placental differentiation at a time when it should be present, with a uniform and unusual development of the villi over the whole exterior of relatively large chorionic vesicles, is decidedly significant and has often been found to imply the presence of hydatiform degeneration. The same thing is true of a very irregular distribution of the villi, or of uniformly distributed fusiform enlargements on the villi and of the loss of the dull appearance of their cut surfaces, as seen under the binocular. As soon as the stroma becomes hydatiform, and even before liquefaction is present, the cut surfaces of hydatiform villi look somewhat shiny and waxy or, perhaps better still, paraffine-like, as in the specimen *in situ* shown in figure 21. A bluish tinge always is present, and this appearance is very characteristic. However, how easily a specimen of hydatiform mole

can be recognized by examination with the binocular alone necessarily will depend also upon the condition of the specimen. If the villi are matted, glued, or macerated, not only the early hydatiform changes but even fairly advanced ones often are masked so completely that recognition is difficult or impossible without histologic examination.

In many early specimens the diagnosis could be made at sight from a histologic preparation under low magnification, even when it was impossible to make a diagnosis by examination with the binocular microscope alone. What makes this possible is not, as has been generally assumed since Marchand's epochal work on chorio-epithelioma, the appearance of the syncytium or that of the Langhans layer or of the trophoblast, but the changes in the stroma which precede those in the epithelium. The evidence in regard to this matter is overwhelming, and in the early stages when the stroma already has been altered, it often is impossible to tell whether the epithelial development is normally or abnormally active. Moreover, in spite of Marchand's statement to the contrary, extremely large cysts often have but a single smooth layer of epithelium. This has been asserted repeatedly by other investigators also. The two layers of epithelium are not by any means always present and, while there is no agreement in the matter, the opinion seems to be that the grade of epithelial proliferation can not be used as a criterion for the determination of the presence of hydatiform degeneration. Menu said that the presence of marked epithelial proliferation was emphasized early by Müller (1847), Ercolani (1876), Franque (1896), and Owry (1897); and according to Pazzi (1908<sup>3</sup>), Ercolani and Polano altogether denied the existence of connective tissue in the hydatiform mole. The same thing was asserted by Sfameni (1903), who claimed to have found further evidence of the exclusively epithelial nature of the hydatiform mole in 1905. According to Sfameni the hydatiform mole does not result from a modification of existing chorionic villi, but from an entirely new growth which is wholly epithelial in character! But this opinion, which was accepted also by Niosi (1906), seems to exist among Italian writers only.

Although Durante (1898) represented extremely long syncytial buds, he nevertheless found (1909) epithelial proliferation present only where certain vascular changes were present. Winter (1907) stated that the condition of the epithelium varies greatly, and Falgowski (1911) emphasized that he could not demonstrate the presence of an increased epithelial proliferation or of vacuolation of the syncytium. Aman (1916) also found that epithelial proliferation may be wholly absent. Ballantyne (1913), on the contrary, found epithelial proliferation "so well developed that it suggested that it is an essential process in the formation of the mole." Ballantyne further likened hydatiform degeneration to edematous growths and emphasized that both really are epithelial new growths. This opinion is accepted also by de Snoo (1914), who regarded the hydatiform mole as a neoplasm of the trophoblast with secondary changes in the stroma.

There is no agreement at present as to whether the epithelial changes are primary or secondary. As is well known, Marchand (1895)—and Müller, Ercolani, and Langhans long before that—regarded the epithelial changes as primary, but

most investigators seem to have come to the opposite conclusion. Some share the opinion of Schroeder that hydatiform degeneration points to a stimulus resulting in hyperplasia of the entire chorionic villus. Nor is there agreement as to what the initial changes are. Durante (1909) regarded the presence of vessels with an imperfect endothelial lining and with thick infiltrated walls as the initial lesion in hydatiform degeneration. These changes were noted by him, especially in trunk villi, and epithelial proliferation was most evident where the vascular lesions were most pronounced. Durante further stated that the chain form of the hydatids is due to the fact that the vascular lesions occur at intervals along the villus. Unfortunately, the structure of long hydatiform villi does not confirm such an explanation nor Durante's conclusion that the hydatid cavities within the villi result from dilatation of the capillaries. Many investigators report the early disappearance of the blood-vessels, a phenomenon which some regard as secondary and others as primary to the death of the embryo.

In the course of this investigation a villus with a normal stroma and normal vascularization never was found to have undergone true hydatiform degeneration, but one with a normally active epithelium—both Langhans layer and syncytium—often was truly hydatiform. That is, it not only was watery in appearance, but also fusiform or globular even in external form. In fact, Marchand (1895) himself found that “Das Epithel welches die Zotten und ihre Anschwellungen bekleidet zeigt ein sehr verschiedenes Verhalten.” Yet even to-day, the feeling on the part of many seems to be that unless a marked hyperplasia of the Langhans layer and of the syncytium is present the condition is not one of hydatiform mole. This position seems to me to be untenable for, as Marchand himself said, the change in epithelium usually is least in the young villi, and he should have added it is unrecognizable in the early stages and in young conceptuses. A perusal of the literature descriptive of the actual cases leaves little doubt upon this point, and a careful study of the advent of the earliest recognizable changes in hydatiform mole is absolutely convincing. The earliest recognizable, even if not the incipient, changes occur in the stroma and in the vessels—and not in the epithelium. In passing, it may be noted that although Marchand stated that the change in the epithelium is primary, he nevertheless somewhat contradictorily added that the most important fact is the degenerative change in the stroma of the villi.

Although not applicable to what I have come to regard as the incipient changes in hydatiform degeneration, it nevertheless is true that the stroma often, if not always, quite early becomes hydatiform—that is, glassy or clear, though not necessarily watery. Moreover, the villous vessels often degenerate or disappear completely at a very early stage. It is exceedingly difficult to make any definite statement as to what is typical regarding the epithelium. This has been said by others also. Indeed, this necessarily follows from the fact agreed to by every one, that histologically there is no true line of demarcation between the ordinary benign hydatiform mole, the so-called destructive benign (?) hydatiform mole—whatever its status may be—and the malignant hydatiform mole, or chorio-epithelioma. Such a conclusion alone presupposes the existence of the widest differences in the



condition of the epithelium in the these cases, and that such differences actually exist is beyond question.

Marchand's revolutionary investigation on chorio-epithelioma notwithstanding, the epithelium is not always two-layered, nor is it always thickened, in hydatiform mole. That the epithelium can not always be active beyond the normal follows also from the fact that the proliferative changes in it are subsequent to, even if not necessarily consequent upon, changes in the stroma. Furthermore, like the latter they are gradual in their evolution and may stop or be stopped at any stage of their development. Then, too, the condition of the epithelium depends very largely upon the preservation of the abortus, and this, as is well known, varies greatly. The most striking thing about the epithelium usually is not its thickness, the presence of large masses of trophoblast, or of numerous syncytial buds, but its splendid state of preservation, especially as contrasted with that of the stroma. This is true of all except macerated or degenerate specimens, for the life of the epithelium seems assured as long as there are periodic accessions of fresh blood, which, as the clinical histories illustrate, usually is the case. The stroma, on the other hand, probably not being wholly independent of the contained capillaries, is deprived very largely of its sustenance during, even if not in consequence of, their degeneration. According to some, hydatiform degeneration of the stroma is the result of an accumulation of nutritive products in consequence of the absence of the vessels. Degeneration of stroma and vessels, however, may result from malnutrition due to poor implantation.

The epithelium of the villi often was found single-layered without any syncytium whatever, or with at most a few syncytial buds. Nevertheless, both the syncytium and trophoblast very often show evidences of a marked activity which is not confined to implanted villi or to the epithelium of the villi as a whole, but which may extend to that of the chorionic membrane as well. Surprisingly long, complex syncytial buds, whorls and festoons, as shown in figures 22, 23, and 24, and as said to have been observed by Fränkel, often are present, especially on the villi, although in a few instances fine buds and frameworks of syncytium also were seen arising from the epithelium of the chorionic membrane. This feature (shown in figure 23) has, I believe, not been specially emphasized heretofore, though observed by Clivio (1908).

Mounds formed by the Langhans layer were common, especially on the tips of the villi where they frequently formed irregular masses of small nodules—the “*appendici durate*” of Crosti (1895). These gave the villous tree the appearance of a leafless orange loaded with fruit, only that the trophoblastic nodules are mainly apical, as shown in figure 25. In several instances syncytial buds were found far out on these trophoblastic masses, a fact which is of special, if not of crucial significance in connection with the old question of the origin of the syncytium, for these buds undoubtedly had not been transported there. But however one may regard these things, such appearances as represented in figure 24 are unmistakable, for they show thickenings composed of Langhans cells and garlands of considerable length, portions of which are composed of absolutely distinct cells of the Langhans type, as well as other portions composed of syncytium with every gradation between



the two. Nor do I believe that the assumption that syncytium can resolve itself into individual cells can be used to deny the implication of these facts.

Although hydatiform villi covered by a single layer of rather small cells of the nature of Langhans cells, sometimes without visible cell boundaries, frequently were seen, villi covered by typical syncytium only never were seen. The single layer present, although syncytial in places, suggested Langhans cells rather than the real syncytium. Moreover, since the cells of the Langhans layer usually were smaller rather than larger than normal, it follows from this alone that their proliferation must have been marked, in order to completely cover the enlarged villus, in spite of the fact that the layer remained single-celled. Were this not the case, the extraordinary increase in size which accompanies the formation of large hydatid cysts could not possibly occur without rupture of the covering layer.

Not infrequently proliferation of the epithelium without increase in thickness may manifest itself in another way. The caliber of the villi in the earlier stages of hydatiform degeneration sometimes does not increase much, no thickening of the proliferating epithelium is noticeable, and yet the latter shows marked proliferation. Under these circumstances, the borders of the villi and of the chorionic epithelium may appear extraordinarily sinuous as illustrated in figure 26, and epithelial invaginations from opposite sides rarely meet in the center, as indicated in figure 27, and by fusion completely isolate a portion of the stroma. It usually is in these cases of very sinuous epithelium that the epithelial invaginations sometimes become constricted, leaving a closed epithelial vesicle or a nodule of epithelium attached to a stalk or wholly isolated within the stroma, as shown in figures 28 and 29. All stages in this process of vesicle formation were found, and rarely also extensions of epithelial sprouts as described by Neumann (1897) and others were seen, portions of which had become isolated in the stroma to appear later as typical syncytial giant cells. These facts, too, would seem to throw a sidelight upon the origin of the syncytium for those to whom this question is still an open one.

All these things abundantly testify to the activity on the part of the epithelium in many hydatiform moles, even when thickening of it is absent, but they are of diagnostic value only if present, and I wish to emphasize again that they may be wholly absent or at least unrecognizable in the early stages. Moreover, the degree of epithelial proliferation varies greatly, as illustrated in figures 30, 31, and 32.

Until I am able to learn more about the structure of normal villi in various stages of development, I am not willing to commit myself regarding the incipient changes in hydatiform degeneration. These may be unrecognizable with present methods. However, it is possible to say that in young conceptuses the disappearance of the capillaries, which was regarded as a possible cause for the development of hydatiform mole by Hewitt (1860 and 1861), and also emphasized later by Hahn (1865), Maslowsky (1882), and by others, undoubtedly is a very early and possibly the very earliest *noticeable* change in some cases. Of course, I do not imply that death of the embryo is the cause of this disappearance, as Hewitt held, and I am not ready to say that the vascular change is the very earliest one in all cases. This would imply that hydatiform degeneration under no circumstances can begin before

the capillaries have appeared in the villi. There is some evidence which suggests that it possibly may appear before this time. If so, it would be incorrect to speak of a disappearance of the vessels in such chorionic vesicles, for if the advent of hydatiform degeneration can precede the appearance of the villous capillaries, vascularization of the villi may never occur. In older conceptuses, however, in which vascularization of the villi has supervened, the first recognizable change is the disappearance of these capillaries. Many specimens in which the latter were in various stages of degeneration were examined carefully, and the opinion of Hewitt (1860), that hydatiform degeneration can not arise in villi which have been vascularized, can be regarded as of historical interest only. Different stages in the process of vascular degeneration are represented in figures 33 to 35 inclusive.

Coincident with the disappearance of the vessels, changes in the stroma also are noticeable. Usually it tends to become glassy, the individual nuclei becoming separated farther. The stroma, though apparently solid, is uniformly slightly bluish and vitreous, with well-defined, rather small, pycnotic, pointed nuclei, but with not a vestige of a vessel, though the epithelium is splendidly preserved. The latter may be one-layered or two-layered, and may be accompanied by syncytial buds and trophoblastic masses and nodules. In such specimens the entire picture really is exquisite, and a mere glance through the compound microscope reveals the lack of vessels in the vitreous stroma and the marked differences in size of the sections of the villi.

After these early changes, liquefaction of the stroma usually follows. As is well known, liquefaction generally begins in the interior and first appears in the form of vacuolation; but this vacuolation (which I can not regard merely as an edema) is not intra-cellular but intercellular, and as it becomes more pronounced it really takes on the nature of fenestration. Sections of the whole cross-section of the villi, even though large, may be composed of a series of fenestræ (see fig. 36) separated by exceedingly fine strands of the remaining stroma which may contain remnants of the nuclei. But finally, even the fine trabeculæ separating the fenestræ disappear, and the stage of the watery, old, hydatid condition has been reached. More generally, however, the vacuoles or small fenestræ lying in the middle become confluent at the center of the cross-section of the villus, which then is liquefied completely. As is well known, this liquefaction gradually extends to the periphery as the zone of the surrounding stroma is narrowed in the process. Not infrequently, however, liquefaction of the stroma occurs quite generally throughout the cross-section of the villus and is accompanied by the formation of numerous large cells, the wandering or migrating cells of earlier writers. A few of these cells almost always can be found, and rarely the whole section of the villus is studded with (fig. 37) or even formed by these large, erratic cells which usually lie in fenestræ in the stroma. In other instances a large portion of the sections of the villi may be occupied by them, as shown in figure 38. The presence of these cells in villi regarded as normal has long been known. Their presence in hydatiform moles was noted by Otto, Marchand (1898), Essen-Möller, and by many others. Their occurrence in normal and pathological chorionic vesicles, and their significance are considered

more fully by Meyer (1919). No matter what the condition of the epithelium, or more specifically that of the Langhans layer, the syneytium and trophoblast may be, the above-noted changes in the stroma always are quite typical. They are not the only changes noted, however, and their advent may differ somewhat.

Not infrequently, changes quite comparable to those in the villi occur also in the stroma of the chorionic membrane itself, a fact which has not heretofore been emphasized. Also, it is frequently decidedly glassy; liquefaction may occur here and there and may become complete in the course of time. Hofbauer cells not uncommonly also are present. Among the changes noted in this membrane the disappearance of the vessels is most common and constant, although epithelial proliferation is not rare, as already stated. Moreover, when (as in one of Storch's cases) a hydatiform villus is 15<sup>3</sup> cm. long, one scarcely can doubt that the stroma also must have proliferated—not merely degenerated. Some of the strings of hydatid cysts in a specimen in the Mall Collection have a length of over 10 to 12 cm., and in these cases also one can hardly assume that this increased length in the villi was unaccompanied by proliferation of the stroma. From these things alone it follows that the stroma can not remain passive always, although Gromadzki (1913) concluded that the stroma never proliferates. Vecchi (1906), however, reported an increase in the stroma of the villi, and it will be recalled that Marchand also implied the presence of proliferative changes in the connective tissue when he wrote that they depend on those in the epithelium.

I have never been able to find mitotic figures, a fact which may be accounted for, however, by the presence of degenerative changes due to intrauterine separation and retention of most specimens. Indeed, the failure to find mitoses speaks against proliferation in the stroma no more than in case of the epithelium, in which the presence of karyokinetic figures has been reported by a few investigators only. Yet pronounced proliferation of the epithelium often is present. The failure to find mitotic figures is very likely due to the condition of the material.

Careful scrutiny of a large series of specimens has revealed the fact that the disappearance of the vessels in the villi, in the chorionic membrane, and also in the umbilical cord is centripetal as a rule. However, in many specimens the vessels not only may be present in the chorionic membrane although absent in the villi, but may be very numerous and even engorged with blood. It is difficult to say to what extent the engorged condition of these vessels and of those in the body of the abnormal embryos sometimes contained in these hydatiform moles is due to the propulsion of the embryonic blood before the advancing vascular constriction and degeneration, but I am inclined to believe that the centripetal movement of the process is not a negligible factor.

Although only a few instances of the birth of a living fetus or of a fetus which had reached the later months of pregnancy are recorded in the literature, it now is quite generally recognized that the fetus, though dead and too small for its menstrual age, usually is present. This stands in contradiction to the earlier belief illustrated by the statement of Gierse (1847), that the fetus usually was reported as absent, and that when present (as in the cases of Meckel, Gregorini, Otto, Cruveilhier,

and his own) it usually was less than 1 inch long, even when retained for a period of from 3 to 10 months.

This apparent contradiction regarding the presence of the fetus in hydatiform moles is explained easily by the fact that the cases in the earlier literature are old, far advanced in degeneration, while the more recent literature contains many more in the earlier stages of degeneration. Yet in spite of this fact the earlier opinion survives to the present day, for Graves (1909-10) spoke of "the very unusual presence of a normal fetus inside a mole," and Vineberg (1911) still more strangely held that the presence of a fetus excludes the specimen from the class of true hydatiform moles!

Among the specimens concerned in this report many contained a fetus. This was true of 24.5 per cent of 49 tubal and 64.4 per cent of 121 uterine specimens, including some (9) doubtful cases. In some early specimens the fetus is in a state of excellent preservation. This is what one might expect, for the onset of hydatiform degeneration is gradual and often partial. The condition of the fetus in many of them alone also suggests that its death was secondary to the degeneration.

The fetal length ranges from 1 to 90 mm. in the uterine and from 1 to 80 mm. in the tubal series. Although the average length of the embryo in the tubal series is 12.3 mm., and that of the uterine only 10.1 mm., 58 per cent of the tubal specimens nevertheless were below 7 mm. in length as contrasted with 52.5 per cent of the uterine.

The presence of a fetus with a frequency almost three times as great in the uterine series again indicates that the abnormal conditions within the tubes lead to early death, digestion, and absorption, or at least to dissolution, of the embryo. This fact again points directly to a faulty nidus as causative agent, for if the absence of a fetus is to be laid to primary ovular defects, then one must admit that relatively far more of such diseased ova become implanted within the tube than within the uterus.

Of the many explanations which have been offered for the advent of hydatiform degeneration, none seems to be better established than that of endometritis. This was first emphasized by Virchow (1863), and Lwow (1892) also reported 4 cases in patients under his care in whom lues could be excluded but in whom he held endometritis responsible. Emanuel (1895) was the first, it seems, to demonstrate the presence of cocci in inflammatory foci of round cells in the decidua accompanying a case of hydatiform mole. Veit (1899) also believed that disease of the decidua is the cause of hydatiform degeneration. Veit further stated that Waldeyer, Jarotzky, and Storch also believed that an irritative condition of the decidua is responsible. Stoffel (1905) also found cocci other than gonococci present and says he can not avoid holding endometritis responsible in his case. The association of hydatiform degeneration and endometritis was noted also by Marchand (1895), Oster (1904), and Sternberg; also by Essen-Möller, who reported the phenomenal case of a woman with endometritis, who had aborted a hydatiform mole 18 times in 9 years. Falgowski, on the contrary, concluded that the ova themselves were diseased and argued that hydatiform degeneration should be much more

common if it were due to endometritis. Taussig (1911) also stated that leucocytic infiltration of the decidua is frequently present in hydatiform moles, but insisted that "leucocytic infiltration in the placenta then should not be interpreted as infection. \* \* \* Inflammation and infection should be kept apart." I presume Taussig really meant *infiltration* and infection should be kept apart, and the question then turns upon the structure of the normal decidua and the significance of infiltration for the development of the ovum.

It may be recalled that Marchand (1904) reported the presence of isolated groups of small cells in the normal decidua which looked like mononuclears under low magnification, and which he believed often have been confused with them. But even granting this, and the further facts that the exact histologic changes in the decidua are not fully known, and that it is rather difficult to ascertain just what decidual changes are regarded as evidence of the existence of an endometritis, any one examining a large series of cases of hydatiform degeneration aborted with the decidua can not doubt the presence of marked decidual changes in a very large percentage of them. These changes are not limited to infiltration with scattered round cells or erythrocytes, or to focal accumulation of the same, but often extend to almost complete fibrosis, as shown in figure 39, so that experienced investigators have mistaken the thin, fibrous decidua for a part of the chorionic membrane.

It is true that the existence of these changes in the deciduæ themselves does not necessarily imply that they were antecedent to the implantation of the ovum, but fortunately the clinical histories and material from curettage often supply crucial evidence. From such cases and from the cumulative weight of evidence from the large series of cases here reported, the great majority of which showed decidual infiltration or other changes suggestive of endometritis, the frequent association of abnormal deciduæ with hydatiform degenerations is evident. The fact that the incidence of hydatiform degeneration in the tubal was somewhat higher than that in the uterine series might be regarded as contradicting this relationship, but such is not the case. The mucosa of the tubes at best is an unfavorable nidus for implantation because of the absence of decidual formation alone. Hence, even if salpingitis were somewhat less frequent than endometritis, proper nidification in the tube could easily more than account for the existing differences. Hence the higher incidence of hydatiform degeneration in the tubal series in fact becomes confirmatory of the conclusion that abnormal nidification really may be responsible for the advent of hydatiform degeneration.

The only fact which might be interpreted as indicating that germinal defects primarily are responsible for the development of hydatiform degeneration is the *relatively* higher incidence of the condition in older women. Against this, however, stands the other fact that such women also show the cumulative effects upon the endometrium of age, endometritis and pregnancy. Furthermore, since hydatiform degeneration so often follows one or two normal births or abortions, it would be impossible to find an adequate explanation for the release of the defective ova so often after and not before these events.

I am reminded also in this connection of a case the detailed history of which is fully known. It is that of a robust young woman who successively gave birth

to two moles and then to a normal full-term child and secundines. In this case curettage was done in connection with each mole. Apparently the new endometrium, which had formed after the second abortion and curettage, permitted normal implantation and normal development to progress to term. To ignore the condition of the endometrium in this case and attribute the development of hydatiform degeneration to the successive release of abnormal ova would seem to disregard important facts—especially so since no one has established the occurrence of abnormal ova within the Graafian follicle, a possibility which I do not wish to deny, although Donskoj's report of a case of hereditary mole must surely be taken *cum grano salis*.

That an abnormal nidus may be responsible for the advent of hydatiform degeneration would seem to be indicated also by the fact that the process usually was better developed and more general in the tubal than in the uterine cases. That both endometrium and decidua show astonishing differences in structure under pathological conditions is well known. The entire tubal mucosa, on the other hand, even when normal, forms an abnormal nidus which would affect all portions of early chorionic vesicles somewhat alike, and since, as found by Mall, inflammatory conditions in the tubes predispose to tubal implantation, the higher incidence of hydatiform degeneration in the tubes is easily explained. Nor does the existence of partial hydatiform degeneration argue against such an explanation.

Although Kehrer reported not a single fatality in 50 cases of hydatiform mole, Hirtzman (according to von Winckel) gave the fatality as 13 per cent, Dorland and Gerson as 18, and Williamson as 20 to 30 per cent. Von Winckel (1904) regarded these percentages as entirely too high, however, although Oster reported 2 cases of malignancy out of 15 among cases in which the late results were ascertainable—an incidence of 13.3 per cent. Kroemer (1907) found that chorio-epithelioma developed in 5 out of 15 cases of hydatiform moles, or in 33.3 per cent, but only twice in 3,841 "normal implantations." Daels (1908) says La Torre claimed a malignancy of 64 per cent; de Senarceus one of 28.7 per cent, or 14 out of 49 cases. Fränkel (1910) emphasized that the estimates of the number of cases in which hydatiform degeneration is followed by malignant disease vary greatly, while Robertson (1915) quoted Findley as finding that 16 per cent of 250 hydatiform moles collected from the literature were followed by malignant disease. Briggs, who reported 21 cases of hydatiform degeneration with 2 of chorio-epithelioma or an incidence of malignancy of 9.5 per cent, called attention to the "diminishing ratio in the tendency to malignancy shown by his series."

Findley stated that chorio-epithelioma developed in 131 out of 500 cases gathered by him from the literature, which is an incidence of 26.2 per cent; but, as already stated, most of these cases from the literature are old, advanced degenerations, many of which have been retained for a long time. The tendency to malignancy in these probably can in no way be compared to that in smaller and younger specimens, many of which are aborted entire with the surrounding decidua. Consequently, it need not surprise us that out of 19 cases of this series, in which later reports were obtainable, none were reported as having developed chorio-epithelioma.

Perhaps I may here add a word of caution, however, in regard to a possible change in attitude toward the question of malignancy with a consequent relaxation of vigilance. It is true that out of the 21 cases of Briggs only 2 developed chorio-epithelioma, but it must not be forgotten that Briggs in part was, and I to a far larger extent, am dealing with a different class of hydatiform moles than those upon a study of which the prevailing conception of malignancy is based. Hydatiform moles which continue to grow and which survive for months after the death of the embryo evidently are more vigorous, and hence no doubt also more dangerous than those which are aborted early and spontaneously. Since the latter formed the great majority of all moles here considered, opinions regarding malignancy formed on this basis probably would lead to disaster if applied in practice. Such conceptions would be based upon a totally different incidence than the current one of 1 hydatiform mole in every 2,000 cases. Instead of relaxing our vigilance it would seem wise to increase it, particularly in the cases of so-called spontaneous abortions—the cases in which no ascertainable cause for the termination of pregnancy can be found, especially if the chorionic vesicle is empty or if the embryo belongs in one of the early groups of Mall's classification.

The average age of 36 women aborting hydatiform moles was 31 years. Although I do not regard the *alleged* ages as necessarily the *actual* ones, this average age agrees very well with that of 6 cases reported by Poten, 10 by Donskoj, 23 by Briggs, 6 by Gromadski, and 8 by Robertson. The average age of Poten's cases was 32 years, of Donskoj's 25 years, of Briggs's 28 years, of Gromadski's 29.6 years, and of Robertson's 28.4 years. Pazzi (1908<sup>3</sup>), on the other hand, stated that Briquel placed the greatest frequency of hydatiform degeneration between 20 and 30 years. These averages are so far on the near side of the menopause that one can make liberal allowances for the proverbial disinclination of women to state their exact age, even to physicians, and nevertheless regard the prevailing opinion undoubtedly as ill-founded. If, as Lewis (1906) stated, it is necessary to add only half a year to the average age of a large group of women in order to ascertain the actual average age when considering general social statistics, then everyone will admit that still less allowance than this need be made in the case of women who are speaking to their physicians, knowing that whatever they may say will be regarded as strictly confidential. That it is unnecessary to make large allowances for under-statement of their age on the part of these women is indicated also by the average duration of their married life before aborting moles. This in the case of 29 women was 7.1 years. Hence, if one bears in mind that the average age of first marriages according to Webb (1911) is 25.1 years, one can easily see that the average age of the women aborting hydatiform moles, which was given as 29.6 years, is probably not too low at all, thus confirming the findings of Williamson, who denied that hydatiform mole was especially common near the menopause.

The conclusion that the average age of 29.6 years undoubtedly is near the actual is confirmed also by the fact that a hydatiform mole was the first abortion in 19 out of 41 women, or almost half the number; 12, or almost one-third, had aborted twice, and only 10 had aborted more than twice. But what is still more



confirmatory is the existence of a surprising parallelism between the data on abortion and those on births; 9 of 33 women had given birth to but 1 child, and an equal number had given birth to but 2. Hence over 50 per cent of the 33 women had borne children twice, or less than twice, and only 15, or less than half, had borne oftener than this.

This undoubted evidence of the youth of these women is confirmed still further by the statement of Lewis who, from an analysis of 16,325 first births, found that nearly one-half of them occur between the ages of 20 and 24, almost three-fourths between 20 and 29 years, and that first births are more frequent between 30 and 40 than between 15 and 19 years. I realize, to be sure, that social statistics can not be translated from one country to another without modification, but in such a mixed population as ours this modification probably need be less (rather than greater) than in case of some countries.

The conclusion that the occurrence of but a single birth before the advent of hydatiform degeneration probably implies that such women are relatively young is emphasized still further by the statement of Lewis that in one-third of the marriages in Scotland "the bride had a child when unmarried or was pregnant at the time of marriage," and that 50 per cent of the first births in Scotland occur within 9 to 24 months after marriage. Lewis also gives the average interval between marriage and the first birth in 16,176 first births as 13.54 months, but little more than one year. Since Lewis stated that the interval between the birth of the first and that of the second child is but little longer than that between marriage and the birth of the first child, being only 3.07 years, it is evident that not even those women who had borne two children before the advent of hydatiform degeneration could have been near the menopause. This conclusion is emphasized still further by the fact that in 96.12 per cent of 16,176 fruitful marriages fertility was demonstrated within three years after marriage.

Nevertheless, in spite of the clear implication of all these facts, I wish to emphasize again that since what have been heretofore regarded as hydatiform degenerations were large specimens mainly, it well may be, and according to certain authors it is true, that such cases occur later in the reproductive life of women. Yet it certainly is significant that Findley in tabulating 500 of such cases from the literature found that 275, or 55 per cent, occurred before the thirty-fifth year, and of 36 specimens from the Mall Collection 23, or 63.6 per cent, came from women below this age. It may also be recalled that 78 per cent of Kehr's 50 cases and 90 per cent of Bloch's occurred before the fourth decade.

Fourteen out of 23 cases, or 61.3 per cent of the uterine series, in which the age was given, occurred at or before the thirtieth year, and 18 out of 23, or approximately 80 per cent, at or before the thirty-fifth year. These things abundantly emphasize the conclusion reached by some investigators that hydatiform mole is not *absolutely* more common at or near the menopause. But it nevertheless may be *relatively* more common. That is, the number of hydatiform moles aborted after 40 compared with the total number of pregnancies or births after 40, actually may be greater than this ratio before 40 years.



From calculations based on data given by Lewis the average number of births occurring after 40 years in Sweden, Norway, Denmark, Brunswick, Berlin, Buda Pesth, France, and Scotland is 9.9 per cent. This agrees remarkably well with Bloch's estimate of 10 per cent. But if 77.2 per cent of the cases of hydatiform mole occur below 40, and 22.8 per cent after that year, then it is evident that hydatiform mole nevertheless is relatively more common after than before 40 years, for approximately one-fourth of the cases of hydatiform degeneration would be associated with one-tenth of the births. This would be an increased frequency of 300 per cent above that before 40 years. A similar result would be obtained by comparing Findley's or Williamson's series. Hence, hydatiform degeneration though *absolutely* less is *relatively* more frequent in later life. This fact, however, does not necessarily imply that age in itself is responsible for the increased incidence after 40. A comparison of the incidences of hydatiform degeneration in young and old primiparæ, of good health, might elucidate this question.

These statistics are not in agreement with the prevailing opinion that hydatiform moles are more common in multiparæ than in primiparæ. Indeed, as I understand, they suggest rather that after the first conception, which was normal in a large percentage of these young women, something happened which interfered with the normal development of succeeding conceptions. That, it seems to me, is extremely significant and very suggestive. Here is a group of relatively young women, over 50 per cent of whom had borne but twice and some only once, and then gave birth to a hydatiform mole. While I realize the necessity for circumspection, especially in these matters, these facts seem to me to suggest that something happened to a normal endometrium. Other facts also point in the same direction.

Even if it is not wholly correct, as Findley states, that more cases of hydatiform mole were reported in the last decade than in the previous 14 centuries, it is not unlikely that approximately as many specimens of this condition are contained in the Mall Collection as have been reported heretofore. Moreover, upon the basis of the present rate of accession, a large number of formerly unrecognized cases of hydatiform moles—both tubal and uterine—are donated to this collection annually. This fact, together with others to which attention has been called, ought to stimulate our interest in this problem.

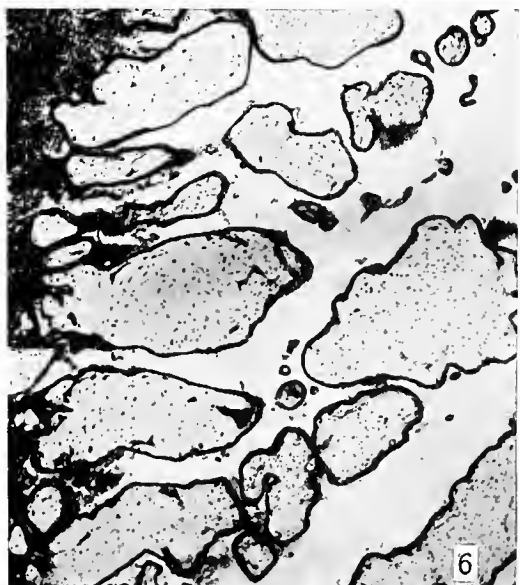
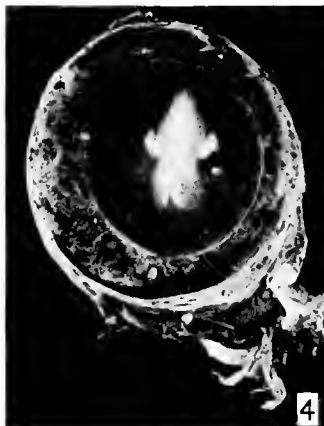
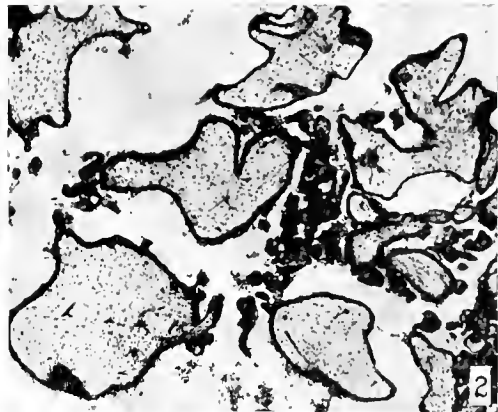
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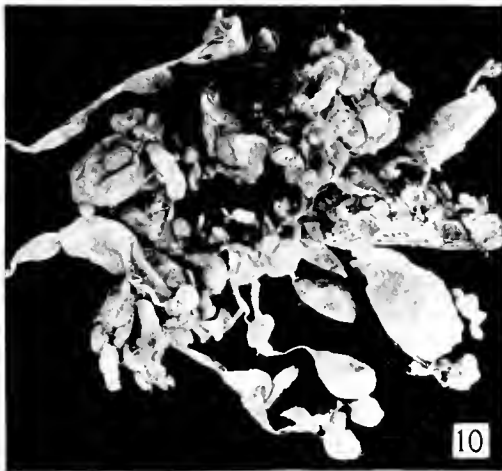
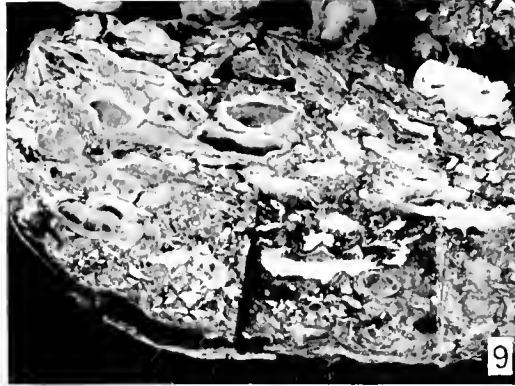
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## LIST OF ILLUSTRATIONS.

- FIG. 1. Cross-section of twin hydatiform chorionic vesicles within the tube. (Specimen No. 825.)  $\times 3$ .
- FIG. 2. Hydatiform villi from same specimen in section.  $\times 45$ .
- FIG. 3. Embryo No. 1771, covered with magma.  $\times 4$ .
- FIG. 4. Cross-section of tube No. 1771.  $\times 2$ .
- FIG. 5. Cross-section of tube from same case.  $\times 4$ .
- FIG. 6. Hydatiform villi from same case.  $\times 45$ .
- FIG. 7. Hydatiform chorionic vesicle *in loco* with the tube incised. No. 2052.  $\times 2$ .
- FIG. 8. Gross appearance of specimen No. 70.  $\times 1.6$ .
- FIG. 9. Gross appearance of specimen No. 323.  $\times 1.75$ .
- FIG. 10. Gross appearance of specimen No. 749.  $\times 1.1$ .
- FIG. 11. Gross appearance of specimen No. 1323.  $\times 1.2$ .
- FIG. 12. Gross appearance of specimen No. 1325.  $\times 1.2$ .
- FIG. 13. Gross appearance of specimen No. 1914.  $\times \frac{1}{2}$ .
- FIG. 14. External appearance of the larger part of the small mass of specimen No. 1926.  $\times 4$ .
- FIG. 15. Embryonic (eyemie) mass accompanying No. 1926.  $\times 4$ .
- FIG. 16. A small portion of specimen No. 1189 still *in loco*.  $\times 6$ .
- FIG. 17. Another portion of the same specimen.  $\times 30$ .
- FIG. 18. No. 2077, an apparently normal specimen, partly surrounded by decidua. Natural size.
- FIG. 19. A portion of the above clearly showing numerous typical hydatid cysts in area in focus.  $\times 3$ .
- FIG. 20. Sections of villi from specimen No. 690.  $\times 50$ .
- FIG. 21. A portion of specimen No. 865 still embedded within the uterus.  $\times 4$ , reduced  $\frac{1}{2}$ .
- FIG. 22. A portion of specimen No. 435<sup>a</sup>, showing a small villus in mid-portion of the figure, which has an exceptionally long syncytial bud.  $\times 50$ .
- FIG. 23. A small portion of specimen No. 962<sup>a</sup>, showing a framework of epithelium arising from the chorionic membrane.  $\times 50$ .
- FIG. 24. Vacuolated masses of syncytium, nodules of trophoblast, and especially garlands composed in part of distinct cells of the Langhans type. Specimen No. 1324.  $\times 300$ .
- FIG. 25. An apical trophoblastic nodule from specimen No. 516.  $\times 6$ .
- FIG. 26. A portion of the chorionic membrane from No. 714, showing corrugation of the proliferating epithelium.  $\times 50$ .
- FIG. 27. A hydatid villus illustrating epithelial constrictions between successive portions. Specimen No. 714.  $\times 50$ .
- FIG. 28. Epithelial vesicles within the stroma. Specimen No. 872.  $\times 180$ .
- FIG. 29. Extremity of an epithelial vesicle. Specimen No. 872.  $\times 300$ .
- FIG. 30. Marked syncytial budding and epithelial proliferation in specimen No. 874<sup>b</sup>.  $\times 50$ .
- FIG. 31. Very slight epithelial proliferation in specimen No. 134.  $\times 50$ .
- FIG. 32. Extremely marked epithelial proliferation on a small, glassy villus from specimen No. 415.  $\times 95$ .
- FIG. 33. Disappearing capillaries represented by large, incomplete curved outlines. Specimen No. 749.  $\times 50$ .
- FIG. 34. Collapsed capillaries in process of degeneration. Specimen No. 712.  $\times 95$ .
- FIG. 35. The degenerating capillaries show as faint lines only. Specimen No. 651g.  $\times 50$ .
- FIG. 36. Fenestrated hydatiform villi among others that are quite fibrous. Specimen No. 651d.  $\times 50$ .
- FIG. 37. A section of villus from No. 510, showing scattered Hofbauer cells.  $\times 300$ .
- FIG. 38. A small portion of a villus from No. 749, showing an accumulation of Hofbauer cells.  $\times 300$ .
- FIG. 39. A portion of a rather fibrous decidua. Specimen No. 874<sup>a</sup>.  $\times 300$ .

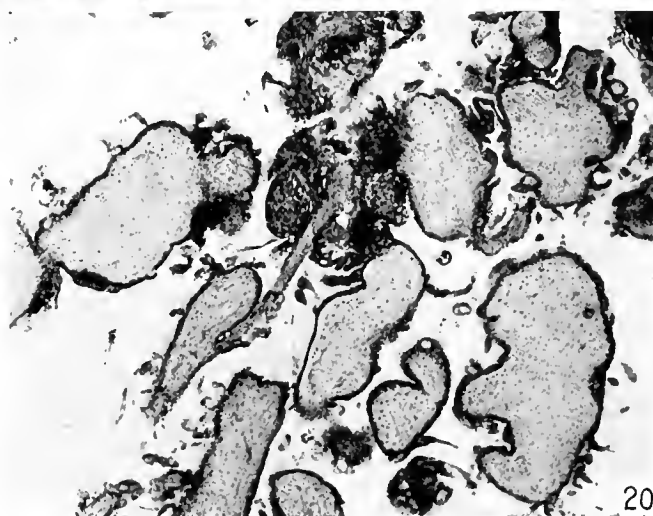
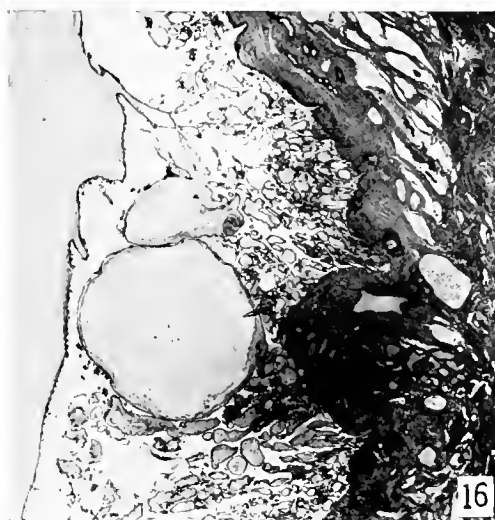




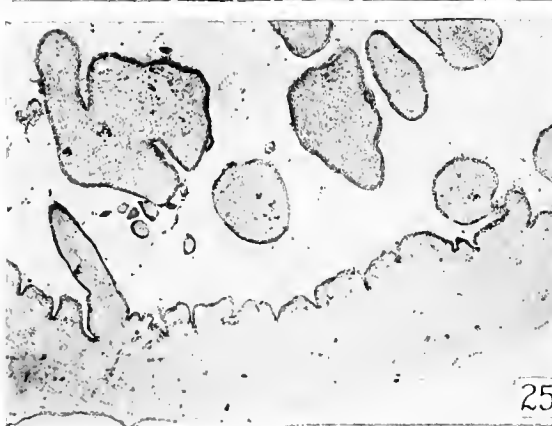
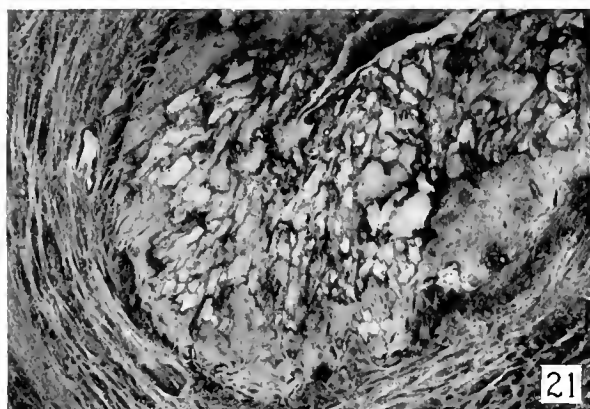




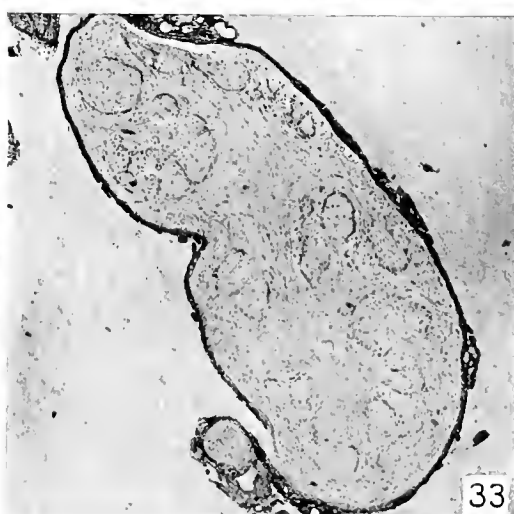
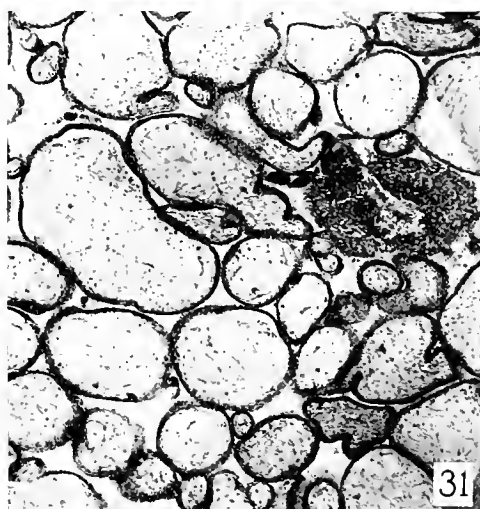
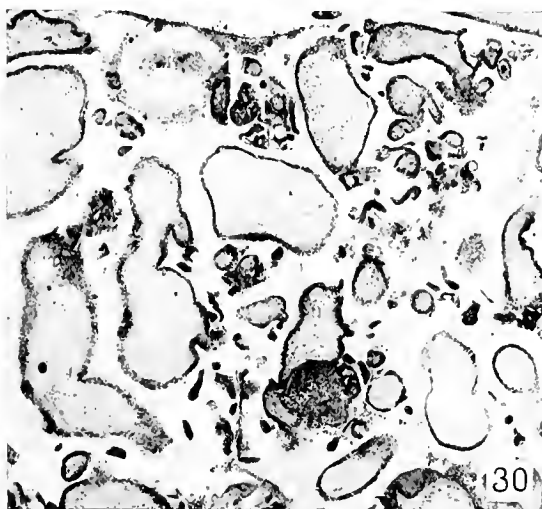
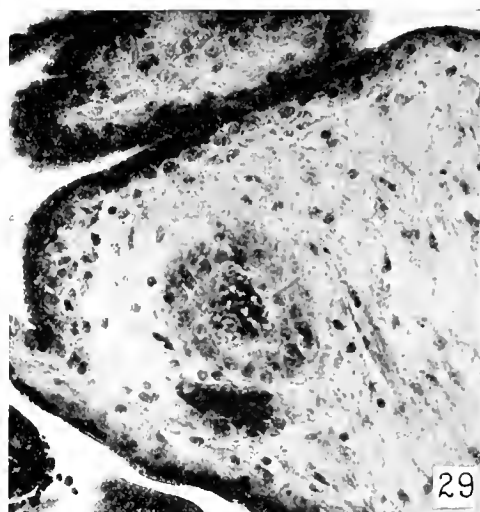
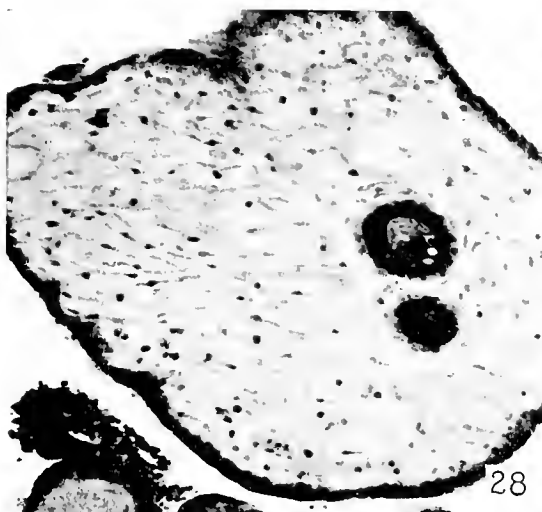




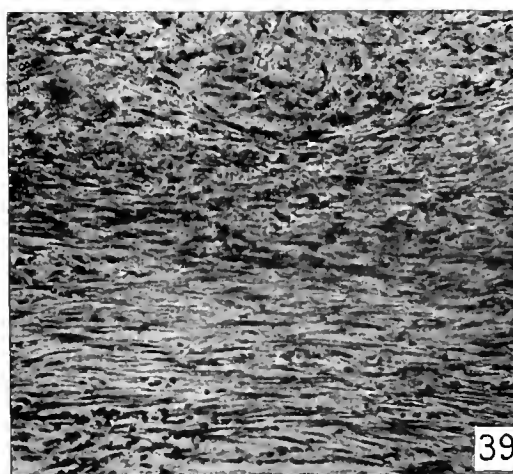
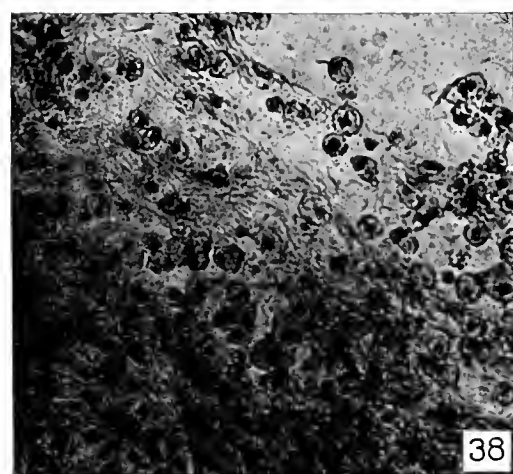
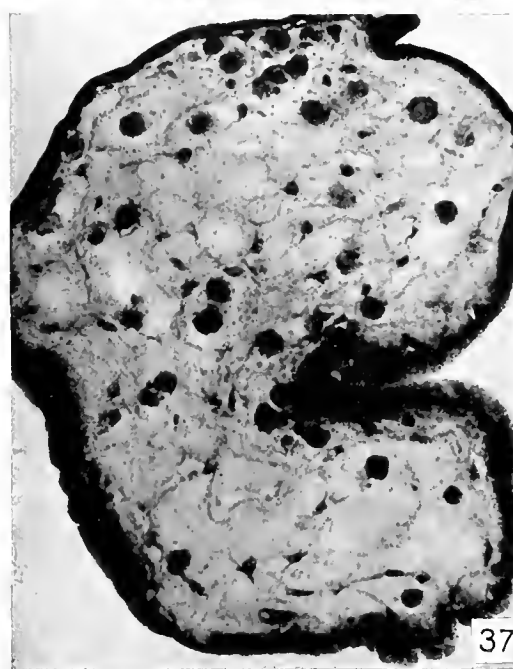
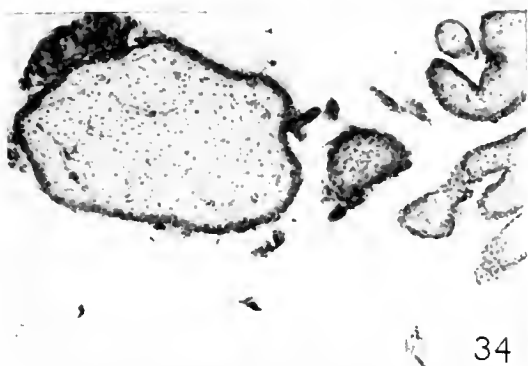
















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CONTRIBUTIONS TO EMBRYOLOGY, No. 41.

A STUDY OF THE DEVELOPMENT OF CERTAIN FEATURES OF THE  
CEREBELLUM.

BY BURTON D. MYERS,  
*Professor of Anatomy in the Indiana University.*

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With six figures.



# A STUDY OF THE DEVELOPMENT OF CERTAIN FEATURES OF THE CEREBELLUM.

BY BURTON D. MYERS.

Through the kindness of Professor von Monakow, it has been my privilege to study fifteen sets of serial sections of brains of different developmental stages,\* selected from very extensive collections in the Institute of Brain Anatomy at Zurich. This study has special reference to certain features of cerebellum. The following report is confined to a brief description of observations on the growth of the Purkinje cells, the growth of the molecular layer of the cerebellar cortex, and the ratio of medullary to cortical zones in cerebellum. For the sake of conciseness, each subject will be considered separately throughout all stages of development.

## PURKINJE CELLS.

In the many investigations on the cerebellum that have been published during the past twenty years, in only a few instances has attention been directed to the Purkinje cells. An examination of the literature covering this limited field reveals the fact that investigators have interested themselves for the most part with the relations, internal structure, and histogenesis of these cells. Popoff (1895) came to the conclusion that they arise exclusively from the deepest cells of the outer nuclear layer. Omer (1899), in a study of the Purkinje cells in the sheep and guinea pig, found that they are derived from non-granular cells of ill-defined contour in the outer nuclear layer. Cajal (1907) directed attention to displaced Purkinje cells, annular terminations around the cell-bodies, and neurofibrils in the protoplasmic aborizations of the cells. No attention has been given by these authors to the determination of the portion of the cerebellum in which the Purkinje cells first make their appearance, or to the possible bearing this may have upon the problem of what, in the cerebellum, is phylogenetically old and what is phylogenetically new. Furthermore, no determination has ever been made as to the number of Purkinje cells that are to be found at the different stages of development, nor have the questions which this point might help to solve received consideration. It was to this untouched field, therefore, that the present investigation was directed.

The Purkinje cells are first definitely demonstrable at the sixth month of intra-uterine life. The cortex of the cerebellar hemisphere of a fetus of this age is shown in figure 1, a drawing with a projection apparatus in which the greatest care has been taken to show every cell of the field in position. A few Purkinje cells are seen along the rather sharp line of demarcation between the nuclear and molecular layers.

\*The series was as follows:

1. Fetus from middle part of 4th month.
2. Fetus from early part of 5th month.
3. Fetus from latter part of 5th month.
4. Fetus from 6th month.
5. Fetus from 7th month.

6. New-born child.
7. Child, 16 days.
8. Child, 3 weeks.
9. Child, 3 months.
10. Child, 2 years.

11. Adult.
12. Microcephalic child, 22 months.
13. Microcephalic child, 2 years.
14. Microcephalic adult, 46 years.
15. Hemiatrophic cerebellum.

The cells have reached their greatest development in the flocculus. Those of the vermis are more uniformly developed than in any other portion of the cerebellum, though the stage of their development is not so advanced as it is in the depth

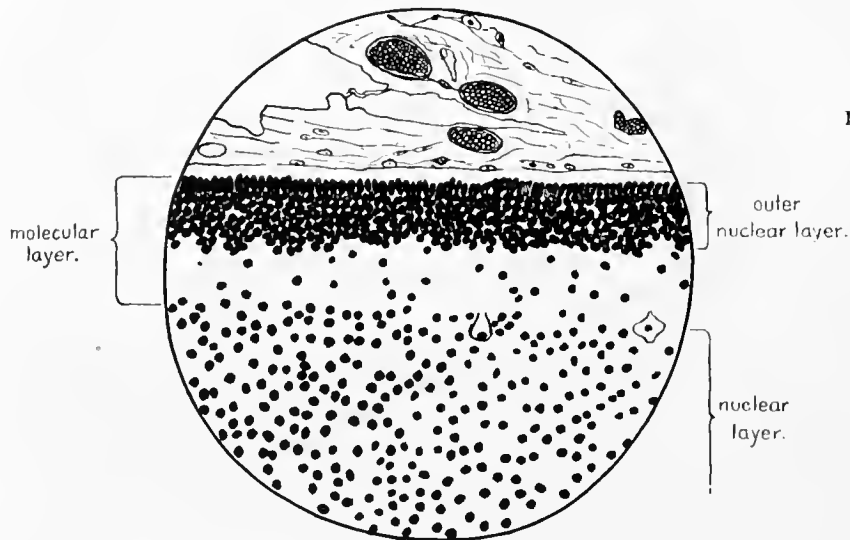


FIG. 1.—Drawing of the cerebellar cortex in which each cell is represented in the field drawn. The most prominent feature of figure 1 is the transitory outer nuclear layer, which occupies a most superficial position in the molecular layer. It disappears at different periods in different animals, corresponding to the age at which myelination in the cerebellum becomes pronounced and locomotion acquired. This outer nuclear layer is probably absorbed by the inner nuclear layer.

of the floccular fissures. It should be mentioned that in a fetus of 6 months the Purkinje cells in the depth of a fissure show a development markedly beyond that of cells more superficially placed. This difference is illustrated in figure 2, a drawing of the contour of the flocculus; *a* and *b* indicate the positions in which the corresponding groups of cells are found. The cells in the depth of the fissure (*a*) show a denser protoplasm, a more definite contour, and better developed protoplasmic processes and nucleus than the more superficially placed cells (*b*). The protoplasm of cells *b*, having no definite boundaries, merges into the surrounding protoplasmic mass. It is less dense, hence the cells appear larger than cells *a*. This same difference is shown in figure 3 for the hemisphere. In the contour drawing *a* and *b* indicate the positions of the cell-groups *a* and *b*. A comparison of the two figures shows how far the Purkinje cells of the flocculus are in advance of those of the hemisphere.

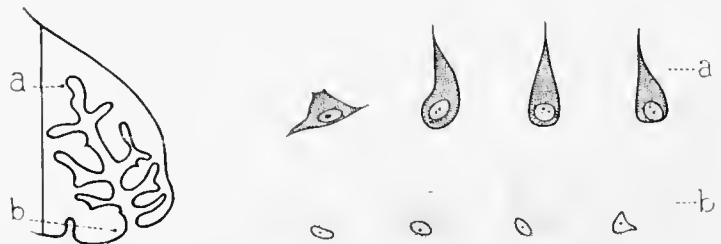


FIG. 2.—A contour drawing of the flocculus. In this drawing *a* and *b* indicate the positions in which the cell groups *a* and *b* are found. The cells in the depth of the fissure *a* show a denser cell protoplasm, more definite contour, and better developed protoplasmic processes and nucleus, than the cells of *b*, more superficially placed.

In the seventh, as in the sixth month of prenatal life, the Purkinje cells of the vermis show a development in advance of those of the hemisphere. Likewise in the new-born the cells of the flocculus are by far the largest, while the cells of the vermis are larger than those of the hemisphere. In both vermis and flocculus the protoplasmic processes are well developed. The Purkinje cells are more numerous

in the former than in the latter. An average field in the vermis shows 36 cells, in the flocculus 22, and in the hemisphere 45.

In the infant 16 days old the Purkinje cells in these three structures have maintained their relative number and size, being largest and least numerous in the flocculus, smallest and most numerous in the hemispheres, and in the vermis occupying an intermediate position as to size and number between the two extremes. An average field in the flocculus shows 19.6 cells (Zeiss Oc. 4, Obj. A. A.), in the vermis 27, and in the hemispheres 34. Upon the theory that the actual number of Purkinje cells in the cortex of a child of 16

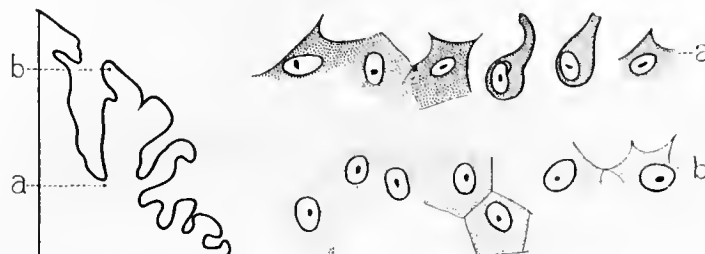


FIG. 3.—A contour drawing of the cerebellar hemisphere; *a* and *b* indicate the position of the cell groups *a* and *b*. The same difference noted between cell group *a*, deeply placed, and cell group *b*, superficially placed in the flocculus (fig. 2), are noted in this hemisphere also.

days is the same as in a fetus of 7 months, we may regard the decrease in the number per field as inversely proportional to the increase in the growth of the cortex.

In table A each number is the average of 20 different fields (Zeiss Oc. 4, Obj. A. A.). A study of this table shows that although at 3 months the number of cells per field is still greatest in the hemispheres, and greater in the vermis than in the flocculus, the ratio is approaching 1:1.

TABLE A.

Age.	Number of cells per field.		
	Flocculus.	Vermis.	Hemisphere.
New-born.....	22	36	45
16 days.....	19.6	27	34
3 months.....	18	20	21
2 years.....	15	17.6	16
35 years.....	14.6	16.9	15.6

If we may regard the number of cells per field as an index of the relative growth of these different parts of the cerebellum, we are led to the conclusion that the cortex of the cerebellar hemisphere increases 100 per cent during the first 3 months of postnatal life, while the vermis undergoes an increase of 80 per cent, and the flocculus an increase of 20 per cent. These percentages are indicative of the relatively greater maturity of the flocculus at birth. At the age of 2 years the number of cells per field is only a little below that of the brain of 3 months, representing a uniform growth of about 30 per cent in the hemispheres, 20 per cent in the flocculus, and 20 per cent in the vermis. It will be observed that the number of cells in the three structures (flocculus 15, vermis 17.6, and hemisphere 16) corresponds almost exactly to the respective number found in the adult (14.6, 16.9, and 15.6); from which fact we may conclude that the cerebellum of a child of 2 years has nearly reached its full development.

Before taking up a discussion of table B let us note briefly the bearing of the results above enumerated. The development of the Purkinje cells in the flocculus, beginning early and progressing more rapidly than in the vermis, is very

unexpected from the old point of view; *i. e.*, that the vermis is the phylogenetically old and the hemispheres the phylogenetically new portion of the cerebellum. It affords valuable evidence, however, in favor of the view recently expressed by Edinger, that the vermis and flocculus are both phylogenetically old. Inasmuch as both sides of the fissura uvulo-nodularis show like development of the Purkinje cells, and as the portion of the cerebellum across this fissure from the flocculus is the representative of the paraflocculus, it suggests the possibility of the paraflocculus, as well as the flocculus, belonging to the paleo-cerebellum.

In the series studied it was possible to first determine the number of Purkinje cells per field in the new-born; from this time on the number remains constant, the apparent decrease being proportionate to the actual increase in surface.

TABLE B.—*Microcephalism.*

Age.	Number of cells per field, Zeiss Oc. 4 Obj. A. A.		
	Flocculus.	Vermis.	Hemisphere.
22 months.....	6.2	9.6	10.5
2 years.....	13.8	11.4	11.8
46 years.....	14.4	14.5	15

Table B deals with microcephalics of various ages, in which the number of Purkinje cells per field was determined as for table A. Upon comparing these specimens with those of corresponding ages given in table A, it will be observed that in the cerebellum of the microcephalic child of 22 months the number of Purkinje cells is about 50 per cent of the normal, as represented by the 2-year-old child given in the preceding table. In the 2-year-old microcephalic the number of cells in the vermis and hemisphere is about 70 per cent of the normal, while the number in the flocculus is about 90 per cent. In the adult microcephalic the number of cells per field is practically normal.

In each of these cases, however, we are dealing with a cerebellum actually smaller than normal, with a total cortical area much less than normal; so that in every case the actual number of Purkinje cells must be below that of the normal cerebellum. In the first two specimens the reduction is due not merely to the actual decrease in cortex, for even where the cortex is present there is a relative reduction of 30 to 50 per cent per field. In the adult microcephalic this decrease in number is directly proportional to the decrease in cerebellar cortex, inasmuch as the number of cells per field is practically normal. This suggests the possibility of a delayed development of Purkinje cells. In microcephalism the cerebrum, as well as the cerebellum, is too small. It is possible that failure of development of the latter is secondary and due to an inadequately stimulating influence from the cerebral cortex.

#### GROWTH OF THE MOLECULAR LAYER OF THE CEREBELLAR CORTEX.

Upon examination of the literature I find that Berliner (1905) is the only investigator who has attempted to determine by measurements the rate of development of the cerebellum at prenatal and postnatal stages. In projection drawings

of mesial sections of a series of cerebella this writer shows that the superficial folding proceeds most intensively during the second half of the prenatal period and the first 3 months of postnatal life. In order to secure more accurate results and a numerical expression of the relation, he measured the periphery of a mesial section in a series of cerebella of different ages, by means of a cyclometer on contour drawings of about 7 magnifications. These measurements show that the period of most rapid growth is from the fifth month of intrauterine to the fourth month of postnatal life.

TABLE C.

Age.	Average thickness of molecular layer expressed in microns.		
	Flocculus.	Vermis.	Hemisphere.
Fetus, sixth month.	106	88	86
Fetus, seventh month	134	97	92
New-born .....	157	167	110
Child, 16 days.....	167	169	123
Child, 3 months.....	258	262	210
Child, 2 years.....	295	314	305
Adult.....	300	317	311

The average thickness, recorded in table C, represents an average of 20 different measurements in each of the specimens enumerated. Measurements were made with the ocular micrometer and reduced to microns. Care was taken to select places for measurement where the cortex was vertically cut. It will be noted, from an examination of this table, that although before birth the molecular layer of the flocculus shows a greater thickness than that of the hemispheres or the vermis, from birth on the greatest thickness of this layer is found uniformly in the vermis. In certain instances, as the 16-day-old child, the thickness of the molecular layer of the vermis and of the flocculus is so nearly the same that the difference is negligible.

TABLE D.—Percentage of growth of molecular layer.

	Flocculus.	Vermis.	Hemisphere.
	<i>p. ct.</i>	<i>p. ct.</i>	<i>p. ct.</i>
From sixth month to birth....	50	90	30
From birth to 3 months.....	64	57	91
From 3 months to 2 years.....	14	20	46
From birth to 2 years.....	88	88	177
From 2 years to adult.....	2	1	2

As with the Purkinje cells, so with the development of the molecular layer, the period of greatest general growth is between birth and the third month of postnatal life. Table D, based upon the preceding, gives the growth in percentages for different periods. It is apparent that the period of most rapid growth in thickness of the molecular layer of the vermis is from the sixth month of intrauterine life to birth; while for the flocculus and hemispheres it is from birth to the third month, though for this period the percentage increase in the thickness of the molecular layer of the vermis is nearly as great as that of the flocculus. It is evident, therefore, that the period of most rapid growth of the layer as a whole is from the

sixth month of intrauterine life to the third month of postnatal life. This result corresponds very closely to that of Berliner, as given above. We have, therefore, in the thickness of the molecular layer an index of the stage of development of the cerebellum, an index which will be applied in the consideration of the microcephalies.

The greater thickness of the molecular layer of the flocculus from the sixth month of antenatal to the third month of postnatal life is additional evidence in favor of Edinger's view that the flocculus, as well as the vermis, is phylogenetically old. It is interesting to note that between birth and two years of age the percentage increase in thickness of this layer of the flocculus and vermis is the same, and just 50 per cent of that in the hemispheres for the same period (see table D).

As in the development of the Purkinje cells, the adult condition of the molecular layer of the cerebellar cortex is practically reached in the second year, the growth thereafter being only 1 to 2 per cent.

TABLE E.—Average thickness of molecular layer.

	Age.	Flocculus.	Vermis.	Hemispheres.
Microcephalic child with spina bifida.	1 year.	161	208	161
Microcephalic child.....	22 months.	205	310	291
Microcephalic child.....	2 years.	277	273	237
Microcephalic adult.....	49 years.	268	371	334
Adult with hemiatrophic cerebellum.	20 years	{ L. 219 R. 259 }	342	{ L. 105 to 292 R. 408 }

The brains reported in table E were all without pathological change except for their small size. A comparison of this table with table C shows that here, in some instances, we have a very great deviation from the normal. In some of the microcephalic specimens, as in the 1-year-old child with spina bifida, this deviation may be interpreted as an arrest of development, inasmuch as in this brain four layers of cells in the outer nuclear layer in the vermis and five to six in the hemisphere still persist. This is a condition which, according to Biach, is normal in a child of 6 weeks. The thickness of the molecular layer of vermis and hemisphere, it will be noted, is about midway between the normal for a child of 16 days and that of a child of 3 months. We have, therefore, a persistence of two conditions—the number of cellular layers in the outer nuclear layer and the thickness of the molecular layer. Each may be considered as an index of development and both speak for an arrest of development in this brain at about the sixth week of postnatal life. This same arrest of development was observed in another case of microcephalism not included in the table.

In the other instances of microcephalism there is no indication of the persistence of a condition normal at an earlier period of development. The outer nuclear layer is entirely absent. The thickness of the molecular layer in some parts, as in the flocculus of the 22-months-old child, is very much below the normal; in other parts, as in the vermis and hemispheres of the adult, it is very much above the normal. In these cases the deviation from normal must be attributed, not to arrest in development, but to an atypical development and an under development as a whole.



In the case of hemiatrophy, the only point of interest is that we have in the vermis and right hemisphere a very marked hypertrophy secondary to the atrophy in the left hemisphere and also to an extent in the flocculus of both sides.

#### OUTER NUCLEAR LAYER.

The most prominent feature of figure 1 is the transitory outer nuclear layer, which at this stage of development (sixth month of fetal life) occupies a most superficial position in the molecular layer. This outer molecular layer has recently been studied in Obersteiner's laboratory by Biach, and also by Löwy. Biach studied the time of disappearance of the layer in the human brain, and found a gradual decrease in the number of layers of cells until the whole disappeared, about the eleventh month. Löwy's study is comparative. He directs attention to the disappearance of the outer nuclear layer in different animals at very different periods, corresponding to the ages at which myelinization in the cerebellum becomes pronounced and locomotion is acquired. He gives a very satisfactory review of the various opinions which have been advanced as to what becomes of this outer nuclear layer, whether it goes to help form the inner nuclear layer or the Purkinje

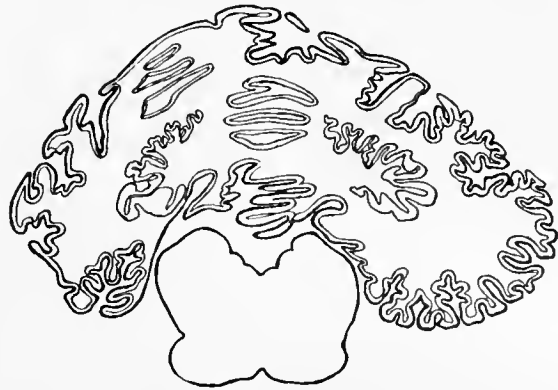


FIG. 4.—Cross-section of the cerebellum of an embryo of 6 months, from the cortex of which figure 1 is drawn.

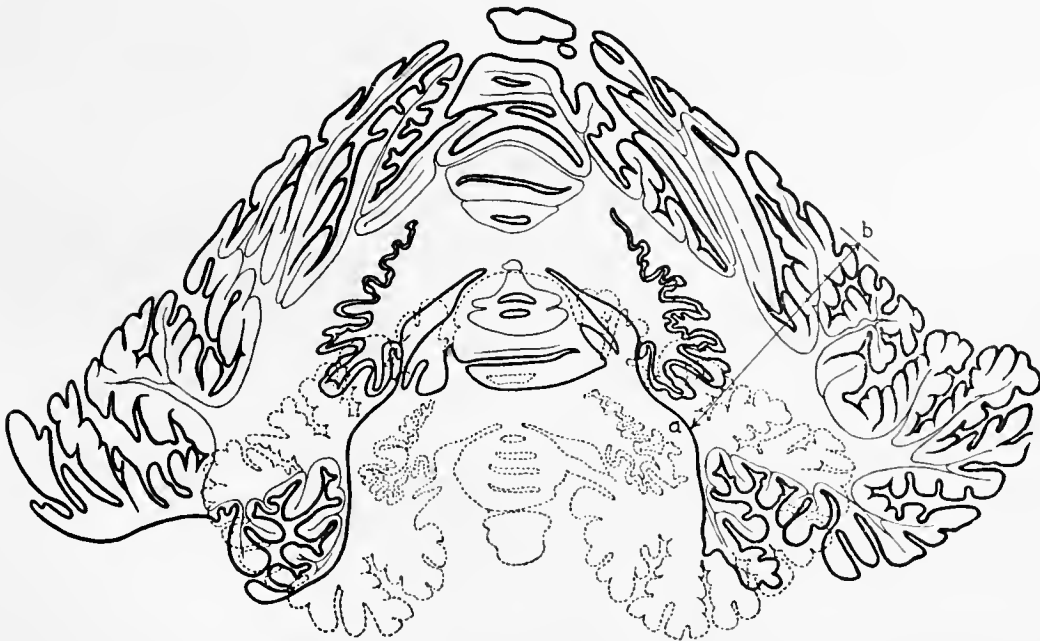


FIG. 5.—Two drawings have been superimposed for convenience of comparison. The outline drawing is from a fetus of 7 months, while the heavy, continuous line drawing is of a 16-day-old child. Figures 5 and 6 show the tremendous increase in cerebellar cortex at the time the outer nuclear layer is disappearing.

cells, constitutes a depot for reinforcement of other layers, or disappears in part. The most general view is that of Cajal, that the disappearance of the outer nuclear layer represents merely a change of position.

In the human brain these cells are disappearing at a time when, as is easily seen in figures 5 and 6, the increase in cerebellar surface is very great. The number of cells in the outer nuclear layer, seen in figure 1, is very striking; but when we compare figure 4 (a cross-section of the cerebellum of a 6-months fetus from the cortex

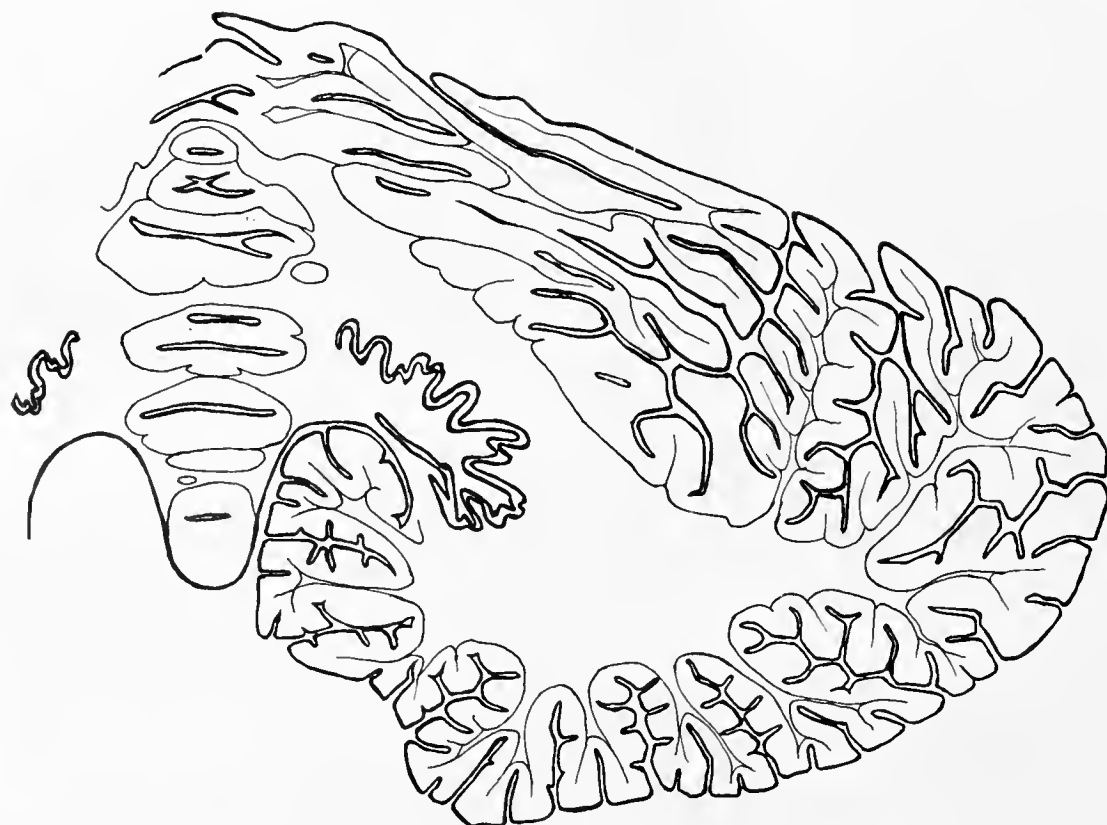


FIG. 6.—Contour drawing of one-half of the cerebellum of a child 2 years of age.

of which figure 1 is taken) with figure 5, it is evident that we have at 16 days a surface at least 10 times that of the 6-months fetus. This means that this outer nuclear layer would furnish to the nuclear layer proper, as present in the child of 16 days, one layer of cells with as much space between adjacent cells as is found between alternate cells in the outer nuclear layer of the 6-months fetus. Physically, the absorption of the outer by the inner nuclear layer is very easy, and it would seem unnecessary to postulate the total disappearance of any of these cells.

#### THICKNESS OF MEDULLA AND CORTEX.

If, in our study of the growth of the cortex, we make a comparison of the thickness of the medullary and cortical portions of the cerebellum, we ascertain that a relation of about 1:1 is maintained, as shown in table F.

In each of the series in which measurements are recorded sections were selected at a level just below the place in which the corpus restiforme passes over into the cerebellum. Measurements were made in each section along the line *ab*, figure 5, passing from the base of the floccular peduncle to meet the surface of the cerebellar cortex at right angles.

In the sixth and seventh months, though medullation is very slight, the medullary and cortical zones are very clearly defined in well-stained sections. The ratio of cortical to medullary field in these series is 3.5:4.5. By birth the ratio of 1:1 has been established between cortical and medullary zones, and this is maintained up to and in the adult. It will be observed that the first two years represent a growth of 100 per cent in thickness of cortical and medullary zones, and that one-third of this growth takes place in the first 3 months.

Though the molecular layer increases in thickness only 1 or 2 per cent after the second year, there is an increase of 15 per cent in the thickness of the cortical zone from the second year to the adult stage.

TABLE F.—*Thickness of cortical and medullary portions of the cerebellum.*

Age.	Cortex.	Medulla.
	<i>mm.</i>	<i>mm.</i>
Fetus, sixth month..	3.5	4.5
seventh month	3.5	4.5
New-born.....	6	6
Child, 16 days.....	6	6
3 weeks.....	7	7
3 months.....	8	8
2 years.....	12	12
Adult.....	14	14

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CONTRIBUTIONS TO EMBRYOLOGY, No. 42.

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FORMATION OF MACROPHAGES BY THE CELLS LINING THE  
SUBARACHNOID CAVITY IN RESPONSE TO THE STIMULUS  
OF PARTICULATE MATTER.

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With one plate.

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# FORMATION OF MACROPHAGES BY THE CELLS LINING THE SUBARACHNOID CAVITY IN RESPONSE TO THE STIMULUS OF PARTICULATE MATTER.

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BY CHARLES R. ESSICK.

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In the course of a study of the processes involved in the localization of an infection within a focus in the nervous system, certain physiological reactions of the cells lining the subarachnoid space have been noted. When active or inert particles of matter are injected into the subarachnoid cavity of a living animal, the cells lining the space hypertrophy, lose their normal attachments, and engage in removing the débris. The importance of such a formation of free-living cells from fixed elements in any process involving destruction and repair in the meninges (infection, hemorrhage, etc.) becomes apparent. The control of cell-reaction promises much in the ultimate therapy of such conditions.

Physiological activity of cells has always been an attractive study, two functions of which may be readily demonstrated in fixed preparations,—*i. e.*, phagocytosis and amœboid wandering. We are accustomed to think of cells as peculiarly fitted to the specialized work in which they are normally engaged; for example, the peritoneal and pleural surfaces are membranes of cells specifically adapted to the free movement of viscera; endothelium of blood-vessels forms a closed tube for conducting the various chemicals used in tissue economy; connective tissue furnishes a supporting framework, and so on. As a corollary to this idea we have to employ a special set of unattached cells to remove products formed during the normal wear and tear of the tissues, and to overcome and remove any noxious stimulants.

The kaleidoscopic changes which take place in inflammation have attracted many observers to the rôle played by the so-called fixed cells and have given rise to a large number of conflicting views. It seems unnecessary, for a clear understanding of this paper, to go into these conceptions in detail. Accumulated evidence leaves little doubt that, under certain conditions, the normal specialized function becomes a secondary characteristic and the more primitive attributes of the unicellular organism become the predominant features. In other words, unless a cell has become too highly specialized the primitive functions of free amœboid movement and phagocytosis may be elicited by the proper type of stimulation in cells which normally are regarded as sessile or fixed elements. The connective tissues have furnished Maximow with a host of cells (polyblasts), normally sessile and in fact almost indistinguishable from their neighbors; such polyblasts under stimulation become amœboid and phagocytic. At times even the fibroblasts may round up and behave toward irritants in the same way that the polyblasts do. Schott (1909), confirmed by Goldmann (1912), showed that the mesothelial lining of the pleural and peritoneal cavity could furnish free-moving phagocytic cells. In exactly the same way, when the destruction of brain tissue occurs, neuroglia cells

pull in their protoplasmic processes and become globular and phagocytic (Alzheimer, 1910). The endothelium of blood vessels has been recognized for a long time as furnishing large phagocytic cells in areas of inflammation. Evans (1915), by offering trypan-blue to the endothelium of the liver, lymph glands, and spleen, has observed the formation of a new circulating mononuclear blood element (macrophage) which buds off the lining of the vessel lumen, but only after prolonged irritation.

To Metschnikoff (1892) we owe the physiological term *macrophage*. This term was adopted because it did not imply a fixed ancestry. An excellent presentation of the biological activities of the phagocytic cells in inflammation of the brain is given by Mertzbaecher (1909). His term *Abräumzell* is much more suggestive in that it calls to mind the attempts at repair which go hand in hand with efforts to nullify the destruction of tissue continuity. This study is concerned with the efforts of the arachnoid membrane towards such removal of foreign material. To include the polyblast of Maximow, the pyrrol-zell of Goldmann, the elasmatoocyte of Ranvier, the endothelioid cells, and Körnschenzellen in the class of macrophages is to simply express a histological similarity combined with a common physiological behavior. The common histological properties are the single nucleus eccentrically placed and a cytoplasm usually reticulated because of the intracellular inclusions. The cells may contain fat-globules, inert bodies, blood elements (either red or white), and albuminous granules, as well as vacuoles. Among their physiological functions are independent freedom of movement and ingestion of particulate matter. In the very first weeks of embryonic life one comes upon such cells fully developed, before any others, to their adult form, first in the placenta and later in the body of the embryo (Essick, 1915). Certainly we are dealing with a universal expression of the need, in the physiological economy of the organism, for cells whose function is to remove the products of tissue destruction. A very good résumé of the literature up to 1909 is given by Schott, who discusses the relation of fixed tissue and macrophages.

For a better understanding of the results of experimental introduction of minute particles into the subarachnoid space with the subsequent formation of macrophages, it seems necessary to give a brief description of the anatomy of the leptomeninges and the mode of preparing specimens for study.

The pia mater and arachnoid may be aptly compared to a living sponge accurately filling the irregularities between the brain and the dura, the exterior surface of which is formed by a closed semi-permeable membrane, while the surface approximating the nervous tissue is perforated by the entrance of the perivascular spaces. Vessels, nerves, and ligaments pass through this layer of tissue without lying free in the cavity. Just as the spaces in a sponge are in free intercommunication, so the cerebro-spinal fluid is normally continuous everywhere. The connective-tissue framework is largely made up of white fibrous tissue, covered by a continuous layer of flattened cells externally, where they form a comparatively simple uninterrupted surface looking toward the dura. The underlying space, known as the subarachnoid cavity, is broken up by anastomosing strands, but the lining presents a continuous unbroken surface of cells except where the perivascular



channels open into it. The spinal cord has been chosen as a place for studying the arachnoid tissue, because of the ease in approaching it experimentally and preparing specimens for microscopic study.

In these observations cats of various ages were used, but the results were uniform. After sacrificing the animals with ether the thorax was opened and 10 per cent formalin was injected immediately into the aorta. A few hours later the bony covering of the central nervous system was removed *en bloc* and the dorsal region of the brain and cord was exposed, care being taken not to rupture the dura. These specimens, partially exposed, were immersed for 5 days in 10 per cent formalin to insure good fixation, after which all of the remaining bone was removed. The dural covering of a section of the cord was carefully removed and the arachnoid separated from the pia as an intact membrane. This was best accomplished by the aid of a binocular microscope, as great care had to be exercised to avoid undue tension on the delicate strands uniting the pia and arachnoid. Eye-scissors were used to cut the trabeculae. The membranes thus obtained were best studied by immersion in a weak aqueous solution of toluidin blue. Permanent preparations were made by the technical methods usually applied to celloidin sections. The conventional microscopic section of the pia arachnoid, in addition to its shrunken and distorted picture, gives one a very limited field to study, as only a small fragment of the rich trabecular system appears in any one specimen. To this limitation is added the fact that the strands are usually cut in such an oblique direction as to render them almost unintelligible. When examined in a dissected specimen the cells clothing the smaller trabeculae are almost completely isolated from their neighbors and furnish a brilliant opportunity for studying them in profile or for noting their cellular contents without confusion. This clearness of picture makes the study of cell hypertrophy and proliferation more convincing because of its comparative isolation, and it approaches more nearly the conditions seen in tissue cultures where cells become amœboid and separate themselves from their normal environment.

The cells covering the membranous expansion of the arachnoid have large, pale, oval nuclei with very indistinct chromatin network (fig. 11). With the ordinary cytological stains the cell-boundaries can not be made out, yet their irregular arrangement may be demonstrated by silver precipitate. Distributed throughout the brain and cord are clusters of closely placed nuclei within the arachnoid membrane. These are well shown in the upper left corner of figure 11. Such areas are irregular in shape, size, and distribution. Histologically they correspond to the arachnoid cell clusters found by Weed (1914, p. 64) in the dura. They represent normal structures and, like the arachnoid trabeculae, become the seat of calcium deposits with the advancing age of the animal. In no sense should they be mistaken for a cellular proliferation in response to a degenerative process. One does not choose by preference the membranous expansion of the arachnoid in studying the formation of macrophages. One meets here the same difficulties that are encountered in the flat serous surfaces, such as the peritoneum. It is hard to eliminate doubt concerning the exact relations of a single cell to the membrane spread out as a flat preparation. Analogous processes can be made out, but not with the

same convincing clearness, as one finds on the thin connective-tissue trabeculae uniting the arachnoid membrane to the pia mater.

The cells clothing the trabeculae are very sensitive to changes in the cerebro-spinal fluid which bathes them. Weed (1917, p. 467) calls attention to this fact by remarking that the "general morphology \* \* \* depends apparently \* \* \* on their physiological state." Particulate matter, either resulting from cell destruction or introduced directly into the cerebro-spinal fluid, calls forth a most remarkable reaction. Inert particles, such as carbon or cinnabar, as well as active matter, such as fragmented red blood-corpuscles or dead leucocytes, initiate morphological changes in the arachnoid cells. The reaction of the cellular membrane to such particulate matter is a slow one and appears to be well under way only after the first 24 hours; dead bacteria may be taken up and removed by the leucocytes before the arachnoid cells show any signs of activity. The most striking results have occurred after stimulation with laked blood, due probably to the fact that it has no toxic effect on the cells and may be utilized by them as food. Partial laking with distilled water was resorted to because the red blood-cells seem to live for some time if injected immediately into the subarachnoid space, whereas laked corpuscles cause a very much more rapid response on the part of the arachnoid cells. This fact suggests a degree of protection against phagocytosis by the living erythrocyte.

The blood was prepared for injection with due precautions to keep it sterile. Twenty cubic centimeters of blood, either homologous or autogenous, were defibrinated by shaking up with glass beads. If massive doses of erythrocytes were desired the defibrinated blood was centrifugalized, and subsequently the isotonicity of the mixture was restored by adding 10× normal concentration of sodium chloride, potassium chloride, and calcium chloride. One or two cubic centimeters could be slowly injected into the lumbar subarachnoid space, or (if a heavy dose were desired) a replacement of the cerebro-spinal fluid over the cord was done in the following manner: A needle was introduced through the occipito-atlantoid ligament and one into the lumbar subarachnoid space. Laked blood was allowed to flow by gravity into the lumbar needle while the displaced fluid made its escape from the occipital region until laked blood appeared. In this way one gets possibly 5 or 6 c.c. of corpuscles around the spinal cord, and if the irrigation pressure is maintained below 300 mm. of water the animal never shows any symptoms referable to the experiment when once it recovers from the anesthesia. The various steps were controlled bacteriologically to rule out a possible confusion with a septic meningitis.

Within 6 hours after the hemolyzed erythrocytes are introduced into the subarachnoid space a full-blown sterile meningitis is in progress; 6,000 to 10,000 leucocytes, composed almost entirely of the polymorphonuclear and transitional variety, are present in a cubic millimeter of the blood-tinged cerebro-spinal fluid. Examined on a warm stage these leucocytes exhibit a most surprising amœboid activity and their cytoplasm is literally stuffed with the small particles of fragmented red blood-cells. At the end of 24 hours the leucocyte count in the spinal fluid has dropped to 2500–1500 per cubic millimeter and in 48 hours has reached the neighborhood of 100. With the decrease of smaller cells from the fluid a new

mononuclear element is found in the cerebro-spinal fluid in increasing numbers. It, too, is actively phagocytic and when studied on the warm stage presents the well-known morphological characteristics of the amoeboid macrophage. The nucleus is large, measuring 7 to 9 microns, and usually has a well-developed nucleolus. The cytoplasm shows a large number of inclusions and vacuoles which take up the main body of the cell and flatten out the nucleus against the limiting membrane. Such inclusions consist mainly of fragmented erythrocytes or pigment granules of reduced hemoglobin. The number of these large cells may reach the neighborhood of 50 per cubic millimeter of cerebro-spinal fluid, withdrawn at the end of 48 hours.

Macroscopic examination of the spinal cord at the end of 48 hours reveals little evidence of the injection of laked blood. A diffuse, pale salmon-pink may be seen through the dura, but unless one were looking for blood the cord might be easily regarded as perfectly normal in color. After five injections of blood, made at 48-hour intervals, a brownish tinge was evident macroscopically, but here again it was not pronounced, except in the immediate site of the injection.

Our chief interest lies in the microscopical appearance of the cells lining the arachnoid space when filled with laked red blood-corpuscles. The normal arachnoid membrane has been beautifully pictured by Key and Retzius, whose technique of preparing specimens for microscopic study was in most respects similar to that employed in these experiments. All of their illustrations show a finely granular protoplasm, becoming coarser around the poles of the oval nuclei. Their figure 1, plate x, corresponds very nearly to the resting normal trabecula which is pictured in figure 3. Their interpretation of this appearance is expressed in the following quotation (p. 127):

"Um die Kerne, besonders aber an ihren beiden Polen liegt ein Haufen von Körnchen, welche theils feiner, mehr protoplasmatisch sind, theils aber grössere glänzendere Kugeln ausmachen. Diese Körnchen kommen fast an jedem Kerne vor, sind aber zuweilen nur sehr sparsam vorhanden, zuweilen aber auch sehr zahlreich, die Enden der Kerne fast verdeckend. Diese Körnchenzone, welche bei jüngeren Individuen im Allgemeinen reichlicher erscheint und als mehr oder weniger veränderter Ueberrest des ursprünglichen Protoplasma zu betrachten ist, streckt sich in verschiedener Entfernung vom Kern auf die Oberfläche der Scheide, sich allmählig verdünnend und verschmälernd, hinaus, bald hat sie eine bestimmtere Begrenzung, bald erstreckt sie sich in verschiedener, zuweilen phantastischer Form, als Seesternarme u. s. w. nach verschiedenen Richtungen, am gewöhnlichsten aber bipolar vom Kern hinaus."

These granules could be stained with rosanilin. Other granulation, similar to fat but not staining so deeply with osmic acid, were observed by these authors in the normal arachnoidal cells. Such appearances are shown in their Taf. x, fig. 3, and Taf. xi, fig. 1.

Specimens dissected from the arachnoid can be best studied in aqueous solutions. A faint stain with toluidin blue will help to differentiate the cell-structures, but the natural differences of refraction and normal color of the fragmented erythrocytes give the most impressive preparations. The first effect of a change in cerebro-spinal fluid is reflected in the protoplasm of the cell. Normally very thin (fig. 1)

so as to be hardly demonstrable, it begins to increase in thickness and the nucleus no longer stands out sharply as viewed in profile (fig. 2*a* and fig. 3). Other cells containing small inclusions of cellular fragments (fig. 4*a*) are found. Their cytoplasm now forms a respectable accumulation around the nucleus and the whole cell projects sharply from the trabecula. The nucleus is more distinctly circular in outline and is seen to occupy an eccentric position in the cell (figs. 2*c* and 4*b*). Still other cells are literally gorged with fragments of erythrocytes, some of which may be almost whole. In this condition (fig. 4*b*) they are about ready to leave the sessile position always occupied and become amœboid.

Histologically, these fixed cells do not differ from the free, round phagocytes which appeared in the fluid tapplings and were identified as macrophages. They are physiologically still a portion of the membranous lining of the fluid cavity, although their attachment becomes more and more restricted. After budding off they tend to become still further distended with erythrocytes and pigment, often reaching a diameter of 16 microns (figs. 6 and 7). Other cells (fig. 8) occur with relatively little vacuolization of their protoplasm and few fragments of erythrocytes. They are smaller (9 to 11 microns) and represent cells losing their attachment while still in the stage represented in figure 3. Their protoplasm is finely granular and dense, showing a paler zone around the eccentric nucleus. The free-moving macrophages gather in clumps (fig. 12) and are most numerous where the débris is greatest. Unless the quantity of matter is very large they quickly store it in their bodies. Occasionally a polymorphonuclear leucocyte, probably representing a dead cell, suffers the same fate as the red blood-cell (fig. 6). The cycle of development may be followed more easily on the trabeculæ, but the cells covering the membranous portion of the arachnoid, as well as those normally identified with the outer surface of the pia mater, undergo the same physiological reactions to the stimulus of the blood (lower portion of fig. 11). Intracellular inclusions are seen clearly enough when looking down upon a cell, but better evidences of the membrane's participation are obtained in cross-section.

The cells of the arachnoid facing the dura mater show similar changes, but in only one instance was a subdural extravasation of blood produced in the region of the spinal cord without the hemorrhage involving the subarachnoid space. Over the cerebral cortex it is rather common to find a hemorrhage separating the dura and the arachnoid membrane. It is then possible to obtain fixed preparations of macrophages in a confined space, with their pseudopodia thrust out for a considerable distance. Such a cell is illustrated in figure 10, showing the characteristic vacuolated appearance of the protoplasm.

This brings up the question of the specificity of certain cells to produce macrophages, and their response to a stimulus applied at a distance. No portion of the arachnoid membrane shows any differences in its behavior towards particulate matter; to produce a response, actual physical contact alone seems necessary. Thus, where the collections of débris are thick almost every cell shows signs of swelling up, while adjoining regions look relatively quiet. This is illustrated more clearly by the different reactions of the cells situated on the two sides of the arachnoid

membrane. If blood is absolutely confined to the subdural space, the layer of arachnoidal cells facing the dura exhibits the characteristics welling-up with macrophage formation, while the layer of cells lining the subarachnoid space, although separated from those of the subdural space by only a very thin layer of white fibrous tissue, remains entirely unaffected. Vice versa, the arachnoid cells composing the membrane looking toward the dura never participate in the reaction towards a stimulus applied to the subarachnoid space. The collection of cells forming the plaques noted above never has been seen to furnish macrophages or even to phagocytize particles of matter.

Permanent specimens stained with the ordinary methods furnish little additional information. Quite often we have all of the inclusions of the cells dissolved out or not counterstained. This gives us a chance to study the protoplasm of the cells as they rest on the trabeculae. The typical foamy structure occurring in the macrophages is shown in figures 2*b* and 2*c*. As ordinarily seen (figs. 9 and 10) this network is the result of the vacuoles and ingested material within the cell. The loose meshwork has been frequently misinterpreted as a sign of degeneration, but it certainly suggests, in these experiments, hungry active cells.

A very natural question arises concerning the fate of the denuded trabeculae and those regions of the membrane which have lost likewise their covering of cells. It is quite easy to convince one's self that the number of cells covering the connective-tissue strands is reduced or, in specific instances, that the entire cellular covering of the trabeculae is gone. Carelessness in preparing the specimen may result in a flaking off of the cells clothing the trabeculae, and undue tension brings about a loosening of the cells or even produces a naked bundle of fibrous tissue. On the other hand, the number of cells per unit of area is by no means constant in the normal arachnoid, and the personal element of interpretation can be exercised to any extent. The efforts to replace cells which have budded off are widely distributed at the time when the production of the free-moving macrophages is at its height. Mitotic figures occur with marked frequency, both among the cells covering the trabeculae (fig. 5) and the membranous expansion of the arachnoid (fig. 11). The localized occurrence of dividing cells corresponds to the areas of particulate stimulation, and in all probability there never exists a true denuding of the surface. During the process of division the protoplasm takes on a denser stain, partaking more strongly of the basic dyes. The protoplasmic bodies of the cells which have not detached themselves close over the gap and very shortly a new division takes place. An actual proliferation of arachnoid cells, resulting in the formation of a regular morula mass, has not been observed in connection with blood stimulation alone, but the combination of infection and blood gave a remarkable picture of this phenomenon.

Experiments were carried out with dilute suspensions of carbon and cinnabar granules. Phagocytosis of inert matter by the cells comprising the membrane could not be expected to be as vigorous as is the case with erythrocytes, inasmuch as the stimulant may be slightly toxic and in no wise can be used for food. Removal of the last traces of such particles involves months. The actual production of

macrophages is not so great as in the above experiments, but one comes upon the same swelling up of the protoplasm and phagocytosis of granules by the cells found on the trabeculæ. The arrangement of the ingested particles tends to be close to the nucleus and consists of the smaller granules. Weed (1917, p. 470) noted, a few hours after injection, "particles of carbon in the cuboidal cells of the arachnoid and similar pictures after the injection of cinnabar." Inert particulate matter which could be easily identified in the tissue has been employed by many observers in the study of the drainage of cerebro-spinal fluid, by injection into the sub-arachnoid space. Quinke (1892, p. 159) remarks:

"Äusserdem fand sich Zinnober in rundlichen oder unregelmässig gestalteten Zellen, die, etwas grösser als Lymphkörperchen regellos verstreut im Subarachnoidalgewebe vorkommen, bald einzelnen bald gruppenweise: und die wohl als Bindegewebszellen von veränderlicher Form anzusehen sind."

Unable to convince himself that the sessile cells took up any of the granules, he concludes (p. 176):

"In den eigentlichen Epithelien der Dura oder Arachnoidea konnte der Farbstoff nie sicher nachgewiesen werden, wenn auch oft genug zinnoberhaltige Zellen der Epithelschicht auffassen. Ebenso wenig fand sich Zinnober in den grossen spindelförmigen Zellen, welche bei jüngeren Thieren die Bindegewebsbalken des Subarachnoidalgewebes bilden, noch in jenen blassen, epithelartig angeordneten Zellen, welche die bindegewebigen Mashenräume dieser Membran auskleiden."

The failure of Quinke was probably the result of waiting too long to study the material—*i. e.*, in periods of a week or more. It appears that the reaction of the membrane is less vigorous after a certain number of cells have become free in the locality; this phenomenon is strikingly illustrated where inert particles are used. Introduction of vital stains into the subarachnoid cavity has not shed any further light on the physiological activity of the lining membrane. Goldmann (1913) makes no mention of vital staining of the meninges. The toxicity of the stain for the nervous system may account for this, as the animals die very quickly after the injection.

The experiments furnishing the material for this paper must be regarded as too acute to shed much light on the fate of these cells which have separated themselves from their normal environment. The use of insoluble inert matter furnished a means of determining this and such a key is found in the work of Quinke. After months these wandering cells may be found, with their cinnabar inclusions, along the carotid sheath to cavernous sinus, along intercostal nerves several millimeters beyond the junction of the sympathetic chain, plexus lumbalis, upper cervical lymphatic and submaxillary lymphatic glands. This shows that the process of migration is slow and dependent on the amœboid activity of the cells themselves. The ultimate disposition of such insoluble matter must be a process similar to the storage of dust inhaled into the lungs. The soluble matter (in the laked corpuscles used) is promptly digested and only the iron pigment remains. No evidence was obtained in support of a view that the free cells after leaving the trabeculæ would again assume their former position.

The excitation of abnormal activities in cells has been variously interpreted and these experiments are subject to the same limitations. The only difference between the same stimulus applied to connective tissue, endothelial walls, or surface of a serous cavity, lies in the peculiarity of anatomical position. On the trabeculae of the slender arachnoid strands the cells are often given but 4 microns to rest upon. There is little surprise, then, that an increase of protoplasm brings about a pendulous appearance. Furthermore, the comparative isolation of these enlarging cells lessens the confusion with neighboring structures as well as permitting a clear view of the cellular inclusions. Material from these experiments, when subjected to the ordinary embedding and section technique, affords very disappointing specimens.

Although the stimulus of injected particulate matter is an excessive one, it points nevertheless to the physiological activities of this cell-membrane forming the walls of the subarachnoid space. Normally, a certain small quantity of débris finds its way into the subarachnoid cavity; this offers an explanation for the finding of a few macrophages as normal inhabitants of the cerebro-spinal fluid. These appear as the large mononuclear cells withdrawn at lumbar puncture. The capacity for developing macrophages occurs in the earliest months of embryonic life and is never lost by the adult pia arachnoid. These findings are comparable with the reactions in other cells of the body, provided the proper stimulus is given. Differentiated mesothelium, such as the arachnoid, peritoneum, and pleura, undifferentiated mesothelium forming the supporting connective tissues, vascular endothelium, and finally neuroglia, may transform into amœboid wandering cells capable of ingesting particulate matter. As a physiological class they may be embraced by the term *macrophage*, and as such are concerned with the reparative processes in the body.

#### SUMMARY.

(1) Particulate matter within the subarachnoid cavity causes the lining mesothelial cells to round up and bud off from their attachments.

(2) As free-moving amœboid elements these cells fulfill in every way the criteria of the class of macrophages, and as such are concerned with the removal of débris.

(3) Normally the same type of stimulus, though in very greatly reduced force, results in the formation of the few large mononuclear cells occurring in the cerebro-spinal fluid.



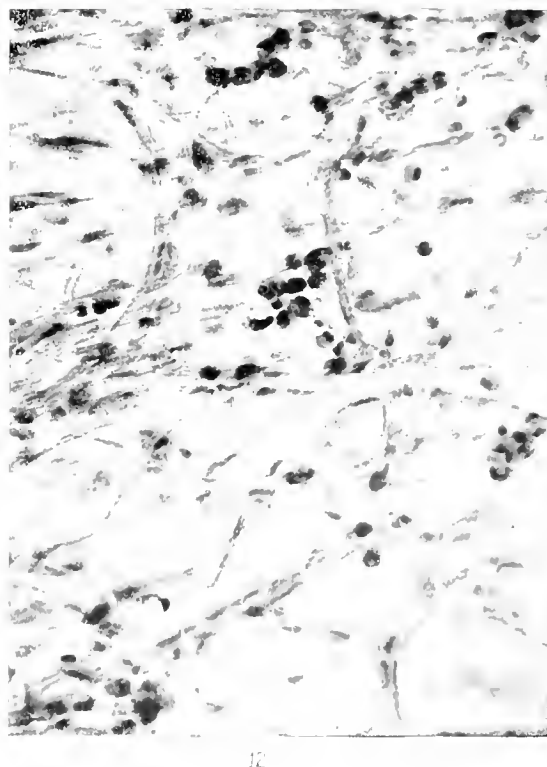
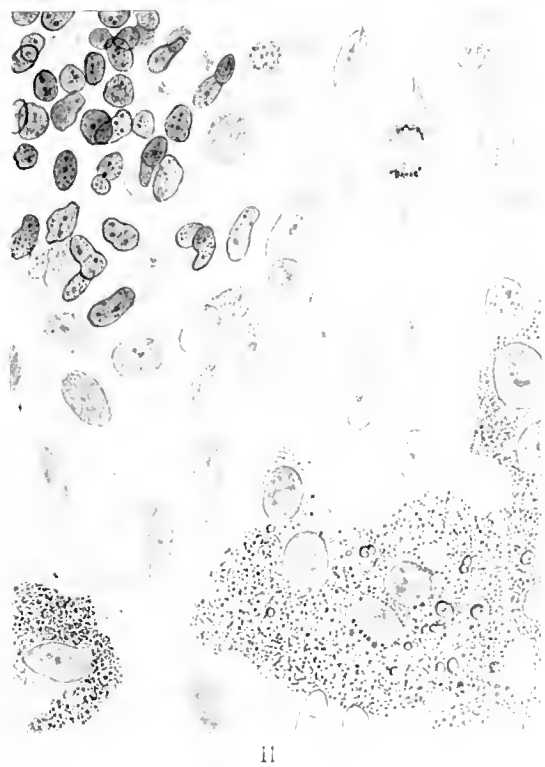
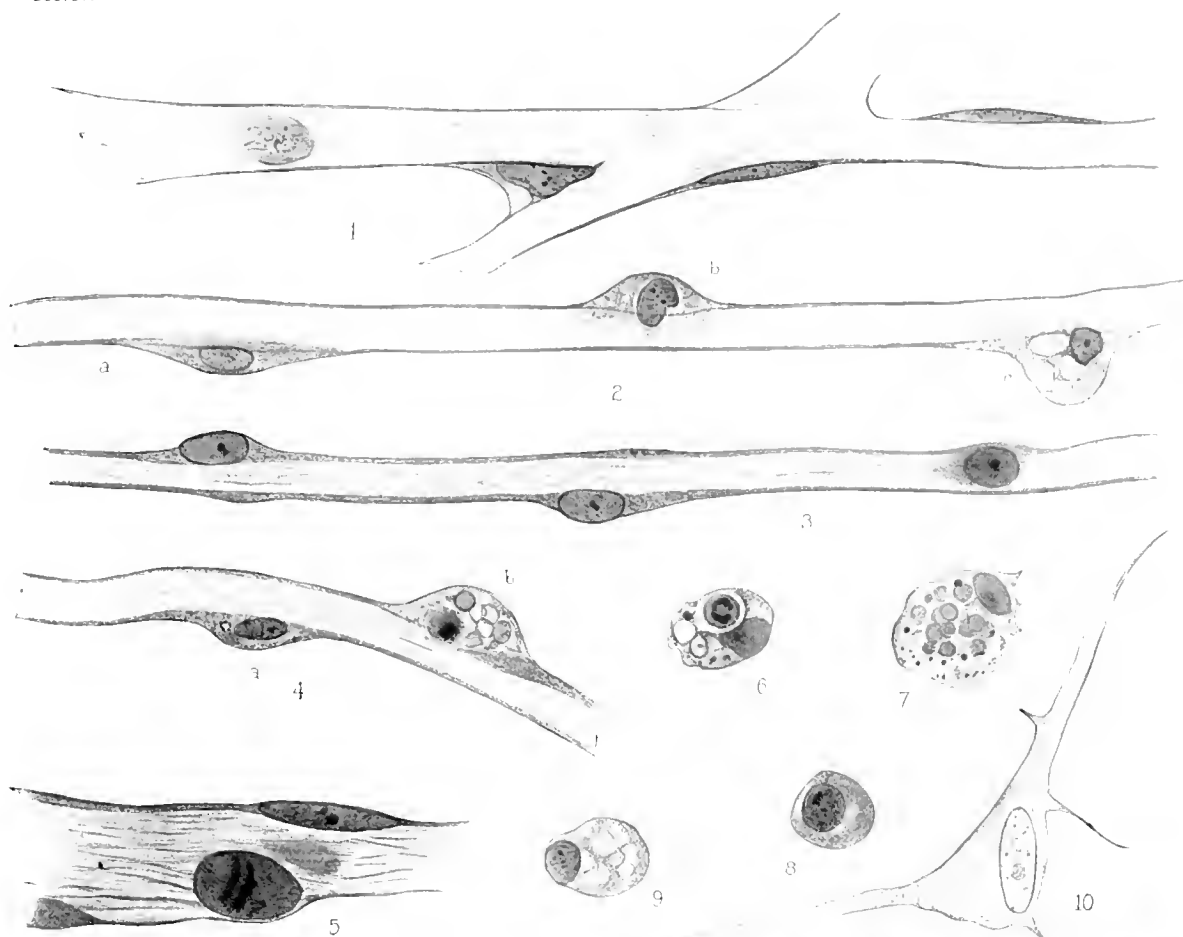
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## DESCRIPTION OF FIGURES.

- FIG. 1. Normal flattened appearance of cells covering the arachnoid trabeculae. Hematoxylin and eosin.  $\times 900$ .
- FIG. 2. Three stages in transformation of arachnoid cells into macrophages occurring after injection of erythrocytes: (a) Primary increase in protoplasm about the nucleus. (b) Beginning formation of a meshwork. (c) Swelling up preparatory to budding off. Hematoxylin and eosin.  $\times 900$ .
- FIG. 3. Initial swelling of protoplasm as shown in preparations stained with aqueous toluidin blue.  $\times 900$ .
- FIG. 4. Phagocytosis of fragmented erythrocytes and blood pigment by cells covering arachnoid trabeculae. Aqueous toluidin blue.  $\times 900$ .
- FIG. 5. Division of arachnoid cell on trabecula. Toluidin blue.  $\times 900$ .
- FIGS. 6, 7, 8. Phagocytic macrophages from subarachnoid space. Stained with aqueous toluidin blue.  $\times 900$ .
- FIG. 9. Macrophage from subarachnoid space. Stained with hematoxylin and eosin.  $\times 900$ .
- FIG. 10. Amœboid macrophage from subdural space. Stained with hematoxylin and eosin.  $\times 900$ .
- FIG. 11. Arachnoid membrane showing phagocytosis of blood-pigment (lower portion of illustration). Reproduction of new elements is shown by mitosis. In the upper left corner appears a portion of an arachnoid cell condensation. Stained with hematoxylin and eosin.  $\times 700$ .
- FIG. 12. Photomicrograph of arachnoid membrane showing clusters of macrophages after subarachnoid injection of laked blood.  $\times 280$ .







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CONTRIBUTIONS TO EMBRYOLOGY, No. 43.

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A HUMAN EMBRYO (MATEER) OF THE PRESOMITE PERIOD.

BY GEORGE L. STREETER,  
*Director of Department of Embryology, Carnegie Institution of Washington.*

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With seven plates and four text-figures.

## CONTENTS.

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	PAGE.
Introduction.....	391
History of the Mateer ovum.....	391
Serial description of sections.....	392
Chorion.....	400
Embryo:	
Embryonic plate.....	402
Amnion.....	403
Yolk-sac.....	404
Angiogenesis.....	405
Comparison with other young human embryos.....	408
Probable age of the Mateer embryo .....	416
Twin embryo.....	421
Bibliography.....	423
Description of plates .....	424

# A HUMAN EMBRYO (MATEER) OF THE PRESOMITE PERIOD.

BY GEORGE L. STREETER.

## INTRODUCTION.

In the literature there may be found descriptions of 16 very young and apparently normal human ova, containing embryos in which the somites have not yet made their appearance. These specimens are all accompanied by authentic clinical data. There are, in addition, 4 presomite specimens which are probably normal, but in which clinical data are missing. When one arranges all of these specimens in their apparent order of development they will be found to fall into three clearly defined groups: First, those in which the primitive groove has not yet appeared; second, those in which there is a primitive groove but no neurenteric canal; and third, those in which the neurenteric canal and medullary groove can be definitely recognized. It has been my privilege to examine a well-preserved, normal specimen which would belong to the second of these groups, and in this paper I wish to present a survey of its main morphological features. The specimen has been temporarily deposited in the Carnegie collection for the purpose of this study, and has been listed in the catalogue as embryo No. 1399; it will be referred to, however, as the *Mateer embryo*, in recognition of the owner, who, appreciating the embryological importance of the specimen, brought it to our attention.

## HISTORY OF SPECIMEN.

The ovum was obtained by Dr. Horace N. Mateer, of Wooster, Ohio, from a fibroid uterus which had been removed by Dr. H. J. Stoll, of Wooster, 11 days after the woman had missed her menstrual period. The patient, a white American, aged 39 years, had been married 17 years. This was her fourth pregnancy, and there had been no abortions or evidence of venereal disease. She is of a prolific family, the mother having had 17 children, and a sister 10 children. The following menstrual history of the case was obtained at the time of operation:

Sept. 10, menstruation began.  
Sept. 12, menstruation ended.  
Sept. 19, coitus.  
Sept. 27, coitus.  
Oct. 8, menstruation expected but failed to appear.  
Oct. 19, hysterectomy.

It may be added that the patient had been informed by Dr. Stoll of the presence of the fibroid condition and warned that in case she became pregnant it would be necessary to remove the uterus. It is therefore probable that she made careful note of such matters and that the above clinical data may be relied upon.

The ovum was dissected out from the uterus and placed in 10 per cent formalin (4 per cent formaldehyde) within an hour after the operation. It was placed upon a bed of cotton and appeared at first to be almost spherical. It flattened out, however, and at the time of cutting was ellipsoid in shape. The specimen was not carefully measured at that time. It was embedded in paraffin and cut into serial sections through its equatorial plane by Dr. B. Harrison Willier, Dr. Mateer's laboratory assistant. In all, 277 sections were saved; 2 passing through the embryo and 6 through the extra-embryonic chorion were lost, making a total of 285 sections. Inasmuch as the microtome was set at  $10\mu$ , this, the shortest, diameter of the chorion may therefore be placed at approximately 3 mm. Forty-nine sections through the region containing the embryo were stained at once in carmine. The remaining sections were mounted on slides and, in February 1916, were forwarded to the Carnegie Laboratory of Embryology, where they were stained by various dyes, including hematoxylin and eosin, iron hematoxylin, eosin, aurantia, and orange *g*.

The 49 sections through the embryo were from the first kept in serial order. The order of the other sections through the extra-embryonic region was only partially preserved, but has since been restored as far as possible. This necessitated the renumbering of the entire series of sections, and throughout this paper the new serial numbers will be uniformly used.

#### SECTIONS THROUGH EMBRYO AND ADNEXA.

In order that the reader may trace the various structures making up the embryo and its adnexa, there will be given here a systematic description of the individual sections. The form of the structures is diagrammatically shown in figures 1 and 2. While the microtome was set at  $10\mu$ , measurements show that a few of the sections were cut irregularly, some being more, others less than  $10\mu$ . Where this occurs note will be made of it.

The chorion as a whole presents the form of a much flattened sphere, and the sections pass through its equatorial plane. The flattened surfaces may be regarded as the two poles; the one to which the embryo is attached we may call the *placental* or *dorsal pole*, and the opposite is the *ventral pole*. These poles are dorsal or ventral as regards the original position of the embryo; that is, the dorsal surface of the embryonic shield is towards the dorsal pole of the chorion, and the ventral surface towards the ventral pole.

## DESCRIPTION OF SECTIONS.

The first 26 sections of the series include only trophoblast and villi. The 27th, 28th and 29th sections traverse the chorionic membrane. In the 30th section one can see the chorionic cavity or exocoelom. The succeeding 13 sections include the narrow interval between the chorionic membrane and the embryo. This space is filled by a fine granular network of coagulum, in the meshes of which a few maternal red blood-cells can be seen. Since the chorionic membrane was not torn, it is probable that these red cells were displaced during the process of sectioning. The knife may have carried them from the intervillous spaces.

Sections 44 and 45 pass tangentially through the extreme rostral portion of the amnion. In section 45 the amniotic cavity makes its first appearance. Dorsally it is loosely adherent to the chorionic membrane.

Section 46 passes through the rostral end of the amniotic cavity and shows the obliquely cut rostral margin of the embryonic plate. An irregular strand of mesodermal cells can be seen everywhere investing the amniotic membrane and embryonic shield.

Sections 47 to 49 penetrate more deeply into the amniotic cavity, showing the folds of the amniotic membrane. Ventral to the amniotic cavity the yolk-sac makes its appearance.

Section 50: A more detailed drawing of this section is shown in figure 16, plate 4. The amniotic cavity is still larger than the yolk cavity. The amniotic membrane is folded so that it can be seen in both transverse and tangential sections. Where cut transversely it appears as a single layer of flattened ectodermal cells closely invested by an irregular layer of mesoderm. Dorsally, loose strands of mesoderm extend toward the chorionic membrane. Around its lateral margin the amniotic membrane bends sharply to become the embryonic plate. In its more lateral portions the embryonic plate consists of one or two layers of cylindrical ectodermal cells. The nuclei for the most part are toward the bases of the cells. More centrally the number of layers is increased to 3 or 4. Owing to the tangential direction of the sections, the middle portion of the embryonic plate appears thicker than it actually is. Ventral to the embryonic plate, strands of mesoderm extend between it and the yolk-sac. The mesoderm seems to be more adherent to the ectoderm of the embryonic plate than to the endoderm of the yolk-sac; between it and the latter there is a series of roomy clefts. The wall of the yolk-sac consists of a rather poorly defined strand of protoplasm with large, round, and oval nuclei, arranged irregularly in two layers. Some of these stain intensely, others are pale. Cell-boundaries can not be well made out.

Within the yolk-sac there is a considerable amount of finely granular coagulum similar to that in the exocoelom. The amniotic cavity is perfectly clear. Apparently the outer row of nuclei represents the investment of mesoderm.

Sections 51 and 52: The space between the amnion and chorionic membrane is bridged by a mass of mesoderm more dense than in the previous sections, so that the sections are now definitely in the region of the body-stalk. Among these cells may occasionally be seen a group arranged in circular formation, so as to form a disconnected endothelial-like space. These spaces are for the most part empty, but now and then they inclose one or more cells. The amniotic membrane is much the same as in the previous sections. The embryonic plate is cut obliquely. The central part is uniform in appearance, showing no evidence of an neurenteric canal. The margin towards the amniotic cavity is covered on each side by a lateral sulcus which demarcates a transitional portion intervening between the embryonic plate and the amniotic membrane. This portion resembles the rhombic lip, to which is attached the tela choroidea in the hind-brain of the adult. About one-third of the distance between this lateral sulcus and the middle line is another groove, less marked, but which seems to be fairly constant throughout the successive sections. In the middle line there is no groove. Between the two sulci on each side the embryonic plate bulges into the lumen of the amniotic cavity, resulting in a longitudinal ridge which can be traced backward to about the region of the primitive groove. In the embedding of the specimen the tissue became brittle and an occasional crack is found crossing the embryonic plate. The mesoderm ventral to the embryonic plate shows points of intimate attachment to the latter, particularly in the lateral portions of the plate. Strands of mesoderm cross from the embryonic plate to the yolk-sac, forming trabeculae, between which is a series of clear, round spaces. Laterally, these spaces are continuous with the cleft that intervenes between the mesoderm and the wall of the yolk-sac, extending about one-quarter of the distance toward the ventral pole. The two layers of the wall of the yolk-sac, the endoderm and mesoderm, are more distinct than in the previous sections. No indication of blood islands is seen in this region. The content of the yolk-sac resembles the granular magma seen in the exocoelom and is perhaps slightly greater in amount.

Section 53 shows very well the attachment between the amnion and the chorionic membrane. The mesodermal cells are closely clustered around the amnion. The apex of the amnion is cut tangentially and so stands out in

marked contrast to the mesoderm. The transition from the amnion to the embryonic plate is clearly shown on the left side of the section, the transitional portion being made up mostly of one layer of ectodermal cells. The embryonic plate is everywhere clearly separated from the yolk-sac by the intervening mesoderm, which at several points seems adherent to it.

Section 54: In this section the amnion comes in contact with the chorionic membrane. The amniotic ectoderm does not show any connection with the chorionic epithelium.

Section 55: In the body-stalk there is seen an endothelial-like space within the lumen of which a cluster of 7 nuclei projects. The mesoderm between the embryonic plate and the yolk-sac is more closely attached to the former than to the latter.

Section 56: (Compare fig. 16, plate 4.) This section is particularly good for showing the relations between the amniotic membrane and the mesoderm. The former is nearly everywhere cut in transverse section except at its extreme tip. The mesoderm is arranged as a membrane, closely investing the amnion and extending a short distance on the body-stalk. Ventrally it extends downward to inclose the yolk-sac, where it can be traced as a separate lamina for about one-half the distance to the ventral pole. Lying free in the exocoelomic space, at the junction of the amnion with the body-stalk, is a small, empty, endothelial cavity. This can be traced only through two sections. It is surrounded solely by finely granular coagulum. The mesoderm between the embryonic plate and the yolk-sac is adherent at many points to both. The wall of the yolk-sac is cut obliquely for the most part. Its cavity now appears somewhat larger than the amniotic cavity. No blood-islands are seen.

Section 57: A very intimate relation exists between the lateral wings of the embryonic plate and the subjacent mesoderm. In the body-stalk, near the tip of the amnion, there is a small mass of cells which apparently are ectodermal and may represent a bud from the amniotic ectoderm, which appears detached on account of the tangential direction of the sections.

Section 58: According to the memoranda obtained from Dr. Willer, two sections through the embryo were lost. On account of the abrupt transition between sections 57 and 58, it would seem probable that the sections are missing at this point. The abruptness is due partly also to the curve in the longitudinal axis of the embryonic plate, so that the plate is cut in this and the succeeding two sections in a markedly tangential direction. In this section the body-stalk is more condensed than heretofore and is fairly well inclosed by a membranous arrangement of the mesoderm. It contains in

its center the tip of the allantoic duct. At one point there is a slight indication of a lumen. The amniotic cavity has become considerably contracted and conforms in a blunt manner to the form of the body-stalk. An intermediate plate still exists between the amniotic membrane and the embryonic plate. This is the first section in which a sharp groove appears in the median line of the embryonic plate—the primitive groove. At this point the ectoderm, mesoderm, and endoderm of the yolk-sac form one continuous mass, which corresponds to the primitive node of Hensen. The extent of this area is exaggerated, owing to the obliqueness of the section. In the ventral part of the yolk-sac a cluster of cells, apparently representing blood-islands, can be recognized.

Section 59: (Compare fig. 14, plate 3.) The body-stalk consists of two portions—a round, more condensed portion surrounding the allantoic duct, and outside of this a triangular area of looser mesodermal tissue which extends up to unite with the chorionic membrane. In the more condensed portion several endothelial spaces can be seen. The allantoic duct contains a lumen. The formation of endoderm, mesoderm, and ectoderm is similar to that in the last section. What appear to be beginning blood-islands can be seen in the ventral part of the yolk-sac.

Section 60: This section was cut 40 $\mu$  thick, otherwise it is much the same as section 59. The body-stalk appears as a very condensed mass and at its center can be seen the allantoic duct with a narrow lumen. The stalk is partly covered with a distinct mesodermal membrane. The amniotic cavity fits close against its ventral wall and is considerably contracted, due to the fact that the lateral intermediate plate lies against the main embryonic plate, thereby reducing the width of the cavity by nearly one-half. Owing to the thickness of the section the details of the fusion between the ectoderm, mesoderm, and endoderm can not be made out. As in the previous sections, however, there is no indication of a neurenteric canal.

Section 61: The transition from sections 60 to 61 appears to be very marked, but is due merely to the thickness of the preceding section, which conceals the change in form which the embryo undergoes at this point. By focusing up and down through the section one can recognize the change from a broad tangential section through the embryonic plate to a thin, narrower, transverse section, sufficient to account for the transition between these two sections. In section 61 the compact portion of the body-stalk is separated from the chorionic membrane by the looser mesodermal tissue referred to in the description of previous sections. The attachment is maintained only by loose strands of mesodermal cells. The allantoic stalk is very



much constricted in this section and consists of only a few cells which are much less compact than in the adjacent sections. Several endothelial spaces can be recognized in the compact portion of the body-stalk. A distinct mesodermal membrane incloses the ventral half of the stalk on each side, spreading over the amnion, whence it continues down over the yolk-sac, constituting the outer of the two layers of the wall of the latter. In the dorsal half of the wall it is distinct from the endodermal layer; ventral to this the two closely fuse and can no longer be distinguished as separate layers. The amniotic ectoderm fits closely against the round ventral surface of the body-stalk and laterally extends downward to a point where it becomes continuous with the transitional portion of the embryonic plate. The embryonic plate proper shows a sharply cut primitive groove, at which point the plate fuses with the endoderm of the yolk-sac. Whether any mesoderm is interposed in this section can not be definitely determined. Lateral to this point there is a considerable amount of mesodermal tissue intervening between the embryonic plate and the yolk-sac, being everywhere closely adherent to the former. It is connected with the yolk-sac by a few slender strands which mark off a series of clear, round spaces, the most lateral of which is continuous with a cleft separating the mesoderm and endoderm from the dorsal portion of the yolk-sac. No endothelial spaces seem to be present in this region.

Section 63: The compact portion of the body-stalk is still farther removed from the chorionic membrane than in the previous section. The allantoic stalk is now somewhat larger, but no lumen can be recognized. A small, endothelial-like ring of cells lies free in the exocoelom lateral to the mesodermal membrane covering the body-stalk. This section passes through the amniotic cavity in a transverse direction favorable for showing the structure of its ectodermal walls. Three distinct regions can be made out—the flattened amniotic ectoderm, the transitional lateral embryonic plate (consisting of one layer of cylindrical cells), and the embryonic plate proper (consisting of two or three layers of cylindrical epithelial cells). At the primitive groove the ectoderm is in contact with the endoderm of the yolk-sac. Lateral to this point there is a considerable amount of mesodermal tissue which has the appearance of flowing out from the lateral portions of the embryonic plate. Strands from this mesoderm extend out to the endoderm of the yolk-sac, outlining spaces similar to those described in the last section. The wall of the yolk-sac in its dorsal half consists of two distinct layers—mesoderm and endoderm. More ventrally the two layers fuse, and in the extreme ventral pole there would appear, in places, to be only one layer, endoderm. At

a few points on the ventral portion of the yolk-sac there may be seen clusters of 3 or 4 nuclei, which possibly represent beginning angioblasts.

Sections 63 and 64: In the body-stalk and in the loose mesodermal tissue between it and the chorionic membrane are several endothelium-lined spaces. In section 64, in the center of the body-stalk, can be seen a well-defined allantoic stalk containing a lumen. The form and structure of the amniotic cavity and its walls are much the same as in the previous section. The relation of the embryonic plate to the yolk-sac is very intimate in the region of the primitive groove. The embryonic plate shows the presence of numerous division figures. The mesoderm intervening between the plate and the endoderm of the yolk-sac is closely adherent to the former. A more detailed drawing of this section is shown in figure 13, plate 3.

Sections 65 and 66: In the loose tissue between the body-stalk and the chorionic membrane is an elongated space, in the lumen of which are a few cells. This is the largest space thus far encountered. The lateral surfaces of the compact portion of the body-stalk are entirely walled in by a mesodermal membrane. In the region of these sections the allantoic stalk is interrupted; at a point where it should be present one sees only the same mesodermal tissue found in other parts of the body-stalk. The amniotic cavity is rapidly contracting; its apex remains flattened in conformity to the ventral contour of the body-stalk. The embryonic plate is much narrower and the primitive groove is still sharply cut. The ectoderm at this point is not so closely adherent to the endoderm of the yolk-sac as in the previous sections. The ventral portion of the wall of the yolk-sac is very much thinned out, and one can not be sure that it consists of more than one layer. In the dorsal portions, however, an outer mesodermal membrane is sharply set off from the endoderm.

Sections 67 and 68: In the body-stalk is an open space in the area where one would expect to find the allantoic stalk, but otherwise there is no trace of that structure. The amniotic cavity is further contracted (see fig. 12, plate 2). The embryonic plate forming its floor still shows the characteristic outlines of a primitive groove at the center. It is thinner than in the preceding sections, consisting of one to two layers of cells, and the middle is no longer in contact with the endoderm of the yolk-sac. The mesoderm between the embryonic plate and the yolk-sac is predominantly adherent to the former, being separated from the latter by a series of spaces similar to those described in the previous sections. In the mesoderm of this region there is no evidence of blood-vessel formation. To the left of the loose tissue, in section 68, intervening between the body-stalk and the chorionic membrane is a group of mesodermal cells which take

part in the formation of a structure that will be followed in the next eleven sections. There is no trace of the allantoic stalk. The primitive groove can still be recognized. The ectoderm at this point, however, is not in contact with the endoderm of the yolk-sac. As in previous sections, the ventral portion of the yolk-sac shows very little evidence of being blood-vessel formation.

Section 69: The amniotic cavity is more contracted and still shows the presence of a primitive groove. The relation of the embryonic plate to the mesoderm intervening between it and the yolk-sac is less closely maintained than in the foregoing sections. In the ventral portion of the yolk-sac is a distinct group of angioblasts, consisting of a strand of about 12 cells. On each side of the strand the wall of the yolk-sac is very thin.

Section 70: The group of mesodermal cells referred to in the last section can now be recognized as arranged in the form of a membrane, cut tangentially. The compact portion of the body-stalk is much smaller, and the only evidence it shows of an allantoic stalk is a doubtful open space. The amniotic cavity is now very small; its ventral floor still has the characteristics of the embryonic plate in contrast to the thin amniotic ectoderm of its roof, as can be seen in figure 11, plate 2. Several angiogenetic areas can be recognized in the ventral portion of the yolk-sac.

Section 71: The body-stalk is now somewhat detached from the loose mesodermal tissue intervening between it and the chorionic membrane. It contains the beginning of the main portion of the allantoic stalk and the tip of the amniotic cavity, the floor of which consists of a small group of ectodermal cells projecting ventrally. The ventral portion of the yolk-sac shows a continuation of the angiogenesis referred to in the last section.

Section 72: Dorsal to the body-stalk can be seen two separate masses, each of which has an average diameter of about the thickness of the chorionic membrane. The one to the left is a continuation of the mesodermic membrane seen in the previous section, and here it can be seen that the mesoderm incloses a solid mass of ectodermal cells of two kinds; a dorsal, paler group, and a ventral, deeply staining group, the two being sharply marked off from one another. The other mass is somewhat less compact and consists partly of mesoderm and partly of cells whose form is better seen in section 73. In the center of the abdominal stalk is the allantoic stalk, sharply marked off and with a clearly defined lumen. Ventral to this is the tip of the amniotic cavity, whose walls are still differentiated in the dorsal amniotic ectoderm and the ventral embryonic plate. The yolk-sac can now be seen at about its greatest diameter, and is spherical

in outline. Its dorsal third is composed of two separate layers, mesoderm and ectoderm. In the ventral two-thirds the layers are so intimately fused that they can not be distinguished; at the extreme ventral pole they have the appearance of a single layer, although the existence of angioblasts in this region indicates the presence of mesodermal elements. One group of angioblasts consists of a round, compact clump of 5 nuclei. The largest group gives the appearance of an elongated oval endothelial space, compactly filled with about 15 nuclei.

Section 73: The character of the two small masses seen in the previous sections, in the space intervening between the body-stalk and the chorionic membrane, can now be clearly made out. The larger one (to the left) consists of an ectodermic vesicle with an average diameter of 0.1 mm. The dorsal two-thirds of its wall consists of a single layer of flattened cells resembling the amniotic membrane seen in the main part of the specimen. The ventral third consists of two or three layers of closely packed cuboidal or cylindrical ectodermal cells. Within the lumen is seen a scant amount of colorless, finely granular coagulum. The whole yolk-sac is surrounded by a more or less membranous and loosely attached layer of mesoderm. The other mass is likewise an ectodermic vesicle surrounded by a membranous layer of mesoderm. It is completely detached from the larger vesicle and differs from it in that its wall consists of a single uniform layer of cuboidal cells. Not including the mesoderm surrounding it, its largest diameter is 0.05 mm. The diameter of its lumen is not quite half that of the larger vesicle. Proceeding to the main part of the specimen we find no trace left of the amniotic cavity in the body-stalk. The allantoic stalk, cut slightly oblique, can be seen with its lumen. The body-stalk is fairly well closed in by a membranous layer of mesoderm. Near its junction with the yolk-sac is a constriction, at the level of which the endoderm of the yolk-sac extends dorsally about half the distance to the allantoic stalk. Upon studying the wall of the yolk-sac one finds the angioblast-formation to be most active at its ventral pole.

Section 74: The larger ectodermal vesicle seen between the body-stalk and the chorionic membrane is cut in a very favorable plane and its structure can be clearly recognized. It apparently represents an amniotic vesicle with a single layer of thin, flattened amniotic ectoderm, and a thick floor-plate of cylindrical embryonic ectoderm, the whole being inclosed by a layer of mesoderm. There is no evidence of a primitive streak. The smaller mass, which is probably a diminutive yolk-sac, shows an incomplete lumen in this section. The body-stalk of the principal embryo shows the allantoic stalk, together with an inverted V-shaped mass

of obliquely-cut endoderm extending from the yolk-sac to unite with the allantoic stalk.

Section 75: The floor-plate of the small ectodermic vesicle lying between the body-stalk and the chorionic membrane is narrower, now occupying only one-fifth of the perimeter; otherwise the vesicle is about the same as in section 74. The smaller adjacent vesicle has disappeared except for a small area of its investing mesoderm. In the body-stalk of the main specimen the allantoic stalk is nearer the V-shaped evagination of the endoderm of the yolk-sac (fig. 10, plate 2). The endoderm appears to be a little thicker in the area of evagination, which is perhaps due to the oblique direction of the section. The ventral portion of the yolk-sac was mechanically injured, as was the case also in the two succeeding sections.

Section 76: The small ectodermic vesicle which we have followed in the preceding sections differs here, in that it consists entirely of thin, oblique cut ectoderm, owing to the fact that the cavity is now contracted. The ectoderm is completely surrounded by mesoderm, which shows a vacuolization-process but no blood-vessel formation. The smaller vesicle has now entirely disappeared. In the main specimen the endoderm has not quite united with the allantoic stalk.

Section 77: The ectodermic vesicle is rapidly contracting and shows an obliquely cut wall surrounded by an irregularly vacuolated layer of mesoderm. In the body-stalk of the main specimen the allantoic stalk is directly continuous with the evaginated endoderm.

Sections 78 and 79: The ectodermic vesicle has now disappeared and there are left only portions of the investing mesoderm. In the main specimen the thickened V-shaped extension of the endoderm of the yolk-sac represents in a clear manner the way in which it evaginates to become continuous with the allantoic stalk, as shown in figure 9, plate 2. In the ventral pole of the yolk-sac numerous foci of angiogenesis can be recognized, the most advanced of which show the presence of completed blood-vessels packed with blood-cells.

Section 80: This section was cut 20 $\mu$  thick. There is nothing left of the body-stalk except its point of attachment to the yolk-sac. The thickened area of the evaginated endoderm and the small amount of mesoderm in the place of the body-stalk can still be made out.

Section 81: Traces of the body-stalk can still be recognized. The ventral part of the yolk-

sac shows very good examples of early angiogenetic foci.

Section 82: All trace of the body-stalk has disappeared. The dorsal pole of the yolk-sac, however, can be readily distinguished from the ventral pole by its distinct endodermal and mesodermal layers, which are separated by a cleft. Also the principal angiogenetic activity is found in the ventral half.

Sections 83 to 91: Ventral and dorsal poles of the yolk-sac can be distinguished. Numerous blood-vessels, partly filled with blood-cells, are found.

Sections 92 to 96: These sections are stained with *erysylechtviolett*, which differentiates very well the cells contained in the early blood-vessels of the yolk-sac. In many of these vessel-forming masses the endothelium-lined lumen and its contained cells are very well differentiated.

Sections 97 to 101: Heavily stained with hematoxylin, eosin, aurantia, and orange *g*. A considerable amount of granular coagulum is present in the yolk-sac and resembles very closely the coagulum existing in the exocoelom.

Sections 102 to 105: Stained in hematoxylin and eosin. The yolk-sac is becoming smaller and the sections through its wall are therefore somewhat oblique. This facilitates the study of the young blood-vessels, some of which exist in the form of a small plexus. In addition to the granular coagulum there is found in the lumen of the yolk-sac a few cells resembling small, mononuclear leucocytes. It is possible that these are displaced cells, as these sections are somewhat broken.

Sections 106 to 110: Deeply stained with hematoxylin, eosin, aurantia, and orange *g*. It is possible that these sections are out of their order and should perhaps have been placed before the preceding four sections. They show considerable angiogenetic activity.

Sections 111 to 115: Stained by the Biondi-Ehrlich method. These sections show very clearly the process of differentiation of the mesoderm of the yolk-sac into endothelium and contained blood-cells.

Sections 116 to 124: The first 4 of these are stained in safranin and light green; the last 4 in hematoxylin, eosin, aurantia, and orange *g*. In these sections the yolk-sac rapidly rounds off and disappears. The coat of mesoderm is thicker in this region and shows well-developed vessels. This point corresponds to the ventral pole of the yolk-sac.

Sections 125 to 277 contain only the chorion.

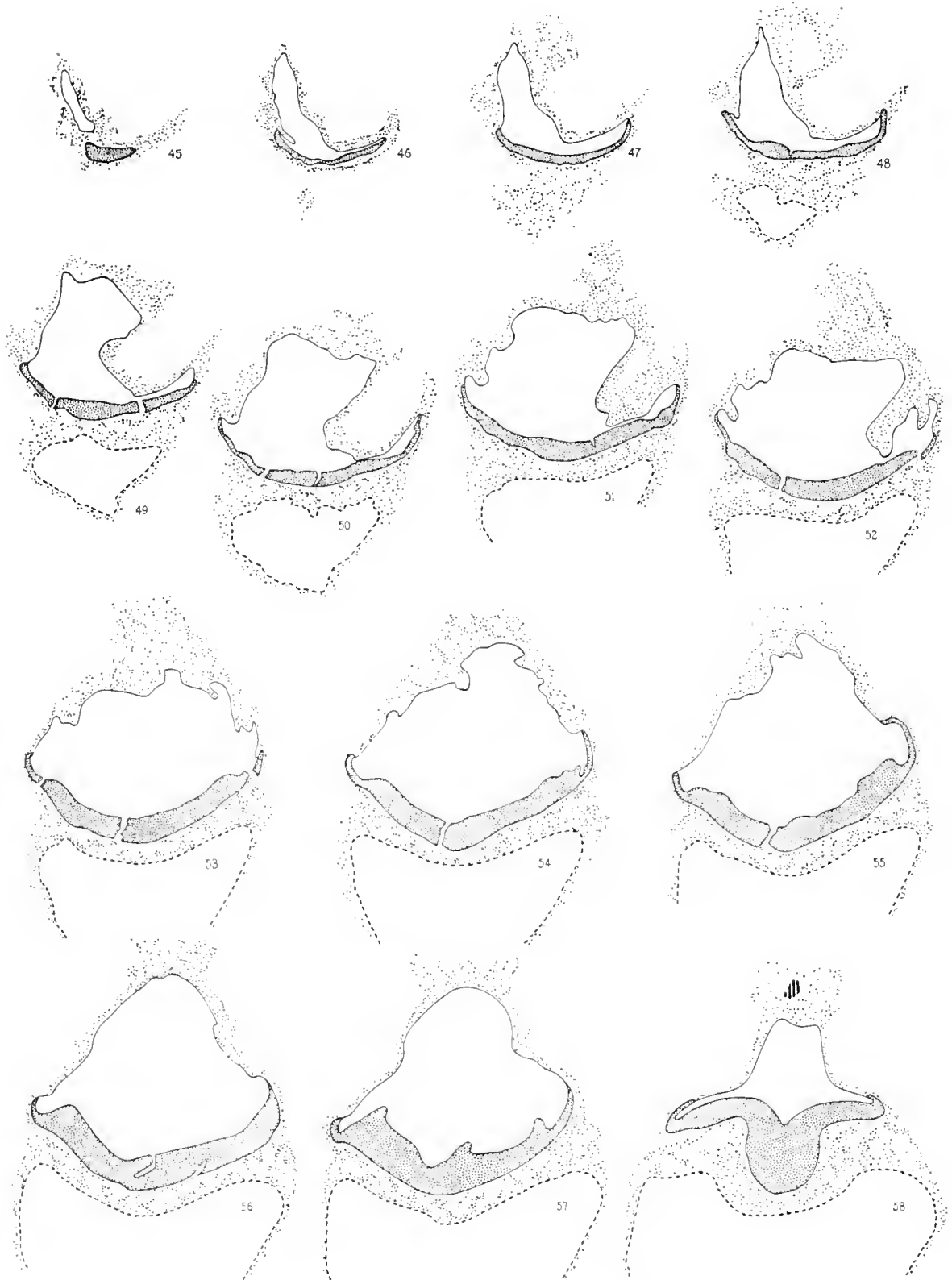


FIG. 1.—Outline serial drawings showing the form of the embryonic plate and its relations to the yolk-sac and the amniotic cavity. The numbers refer to the section number. Enlargement 50 diameters.

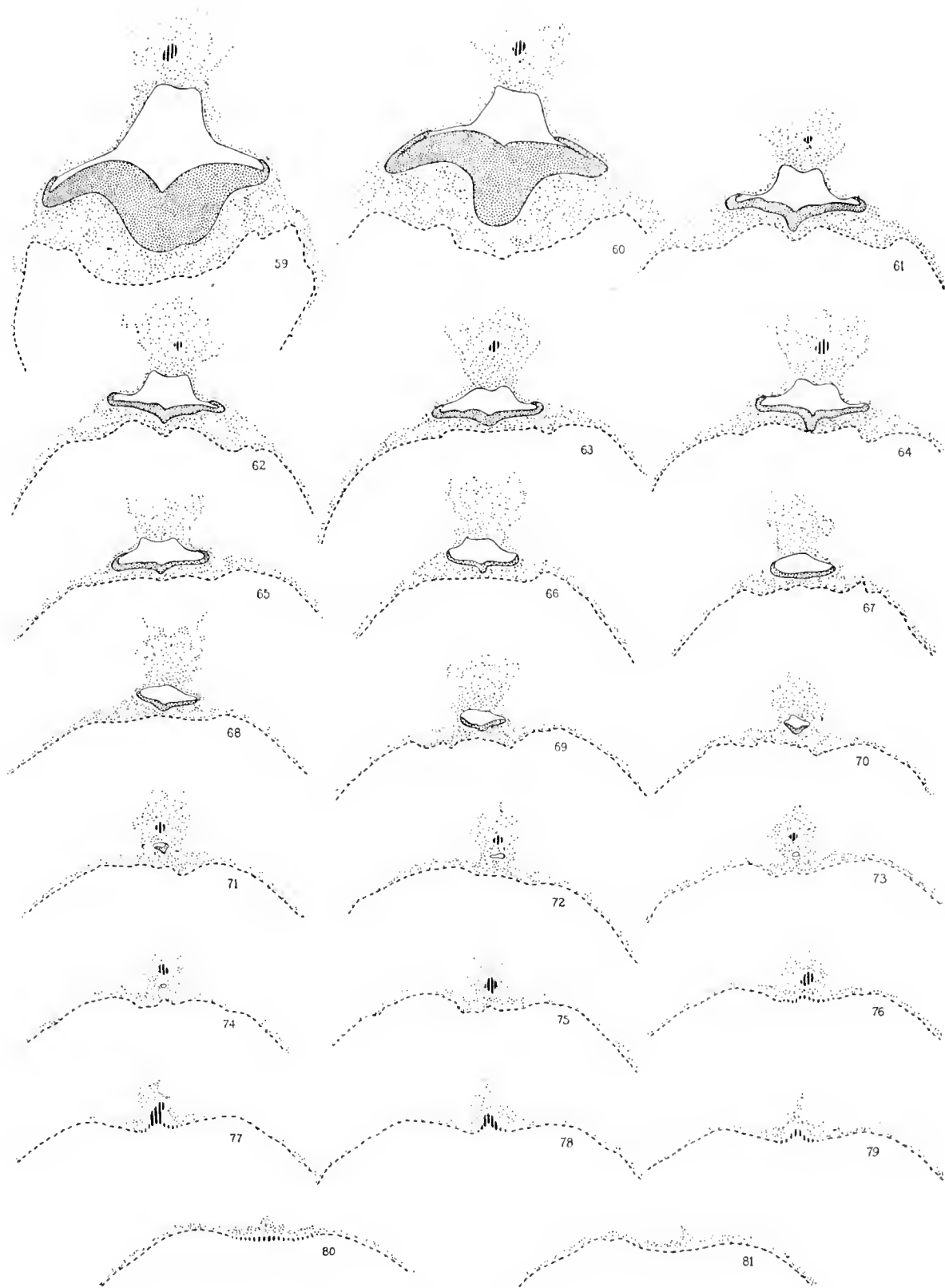


FIG. 2.—Continuation of the series in figure 1. The allantois is shown by heavy vertical lines.

## CHORION.

When the ovum was dissected out from the uterus no drawings or measurements were made of the chorion. It was noted, however, that after being in the fixing fluid, and just before embedding, the ovum formed a flattened sphere. The sections are cut in the equatorial plane and are nearly circular in outline. When reproduced by means of a profile reconstruction they present the form shown in figure 3, which would correspond to a median sagittal section through the whole ovum. This reconstruction was shown to Dr. Willier and it was his impression that the flattening of the ovum, as there represented, is more extreme than the ovum itself actually showed. In case more sections were lost at the time of cutting than the report stated this error would be accounted for. We must therefore introduce a reservation as to the accuracy of the length of the polar axis of the ovum; instead of 3.5 mm., as given below, it may be nearer 6 mm. Measurements taken from the reconstruction and from the individual sections yield the following dimensions:

Outside dimension of entire specimen.....	9.0 by 8.0 by 3.5 mm.
Inside dimensions of chorionic sac.....	6.1 by 5.6 by 2.5 mm.
Length of longest villus (not including cell-column).....	0.8 mm.
Average length of villi.....	0.4 mm.

In the process of dissecting out the ovum practically the whole trophodermic shell was included, some of the areas showing transition into decidua. The arrangement and form of the villi and of the encrusting trophoderm are shown in figure 3. The chorionic membrane seems to be everywhere intact. The cavity contains a coagulum (*magma réticulé*), which in some places takes the form of a compact, finely granular mass, and in others is arranged in finely granular, reticular strands, irregularly meshed. In the sections stained with carmine the magma is barely perceptible, whereas in sections stained with hematoxylin and counterstained with eosin, aurantia, and orange *g*, this substance is quite conspicuous.

The chorionic membrane is made up of a mesodermal and an ectodermal layer. The former presents an entirely different picture from the magma just referred to and there is no evident transition between the two. Owing to the distension of the chorionic cavity the mesoderm is everywhere stretched out as a thin layer covered in by the double-layered ectoderm. The mesodermal and ectodermal layers are for the most part of about the same thickness; in some places, however, the mesodermal layer is thicker. The mesoderm can be traced up into the villi, where it forms their stroma, which is in various stages of vascularization. On examination of the ectodermal layer of the chorionic membrane under higher magnification it can be seen to be made up of an inner, cellular layer (*Langhans layer*), and an outer syncytial layer, the cell-boundaries of which are less distinct and the cytoplasm much more compact and granular. This picture varies somewhat in different portions of the chorion. In some places the two layers are much the same; in others the contrast is quite striking. In some areas the outer syncytial layer shows active vacuolization. Occasionally small syncytial buds are found projecting from the chorionic membrane. The surface of the chorionic membrane is bathed in maternal blood, as is evidenced by the presence of mature blood corpuscles.

Where the villi project from the surface of the chorionic membrane the same general structure is maintained. The villi present a rather uniform calibre and some indication of their shape and manner of branching may be obtained by an examination of text-figures 3 and 4, and figures 6 and 8, plate 1. In general their tips merge directly into the incrusting trophoderm, and where this occurs it is no longer possible to differentiate sharply between the Langhans layer and the syncytial layer. One gains the impression that the former merges into the latter tissue, where the tips of the villi come in contact with the trophoderm, the syncytial layer continuing along its margins.



FIG. 3.—Profile reconstruction showing the Mateer ovum in the median sagittal plane. Enlarged 15 diameters.

The trophoderm completely incrusts the ovum as a trabeculated shell. Although it seems to consist of a uniform tissue, there is considerable variation in its detailed structure in certain areas, these areas merging gradually into each other. This variation applies to the size of the cells, the distinctness of their outline, and the compactness of their cytoplasm. As has been noted in some areas, the trophoderm seems to merge directly with the Langhans layer of the villi and histologically to be a continuation of it. In many places along the periphery it merges into decidua, and much of the trophoderm along the margins gives the appearance of rapid transition into syncytial tissue like that found on the villi. In some cases this transition occurs directly within the substance of the trophodermic mass, and we therefore find small syncytial masses completely imbedded in trophoderm. One also finds more or less detached masses of syncytium scattered everywhere through the irregular spaces of the trophoderm and in the intervillous spaces. These masses present the greatest variety of size and form; some of them seem to be entirely detached, others project into the spaces and are attached only at one end. In some cases they form an enveloping coat for the adjacent trophoderm. They frequently show vacuolization and present a wide variety in the form, number, and character of their nuclei. As a rule the margins of the trophoblastic spaces show a tendency toward the formation of a cell-border which merges into the adjoining trophoderm. In some places this marginal arrangement resembles a thickened endothelium. Along other margins one finds all varieties of transition into syncytial masses. In many respects the conversion of the trophoblastic margins into



syncytial clumps resembles a process of excavation, and in this sense the syncytial clumps would have to be regarded as degenerate trophoblast. In text-figure 4 several varieties of syncytial masses (marked *f*) can be seen in different degrees of formation and in the process of detachment from the adjoining trophoderm. The picture presented by these masses is very suggestive of their retrograde character.

The vascularization of the chorion will be described in connection with that of the embryo and of the yolk-sac.

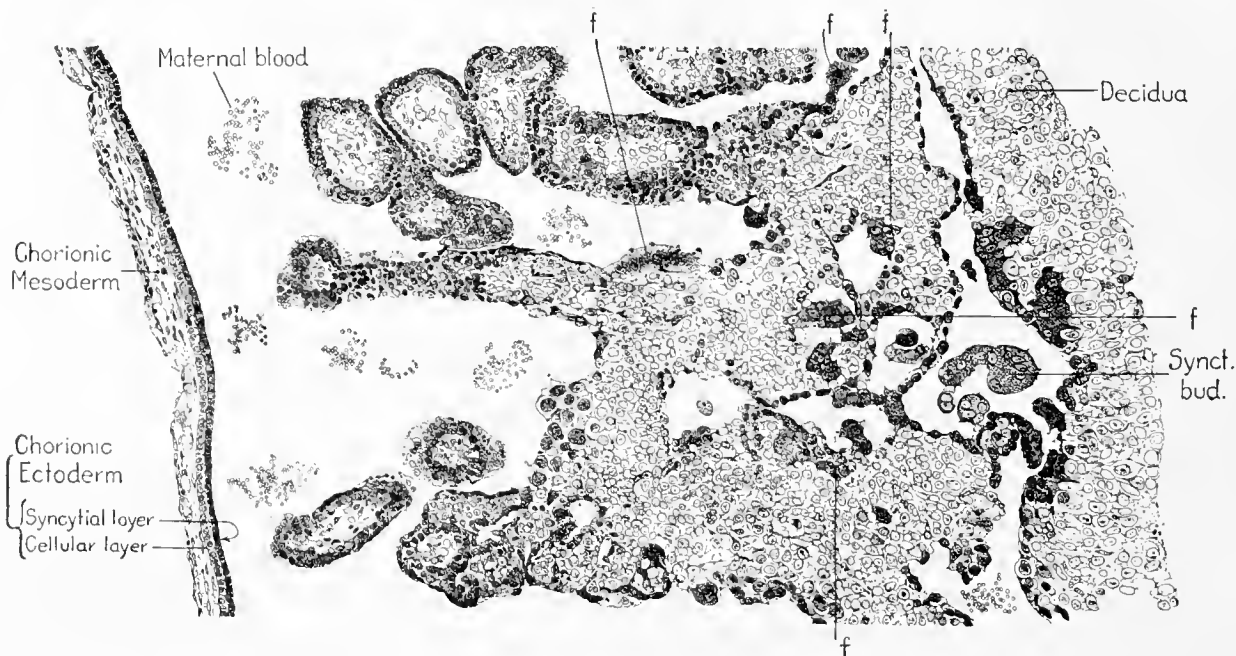


FIG. 4.—Detailed structure of chorionic membrane and villi, showing the transition into trophoblast and decidua. Syncytia-masses in different stages of formation are marked "f." Section 16S, enlarged 125 diameters.

#### EMBRYO.

The form and relation of the embryo and adnexa are shown in plate 1, figures 5 and 6, and typical sections through this region are reproduced in figures 9 to 16, plates 2, 3 and 4, which will be repeatedly referred to. For convenience of description these structures will be taken up separately as the embryonic plate, amnion, yolk-sac, and body-stalk.

#### EMBRYONIC PLATE.

This is somewhat oval in outline. Caudally, it narrows rapidly and is somewhat pointed where it terminates at the body-stalk. In its widest diameter it measures 0.75 mm.; it is 1 mm. long in its median axis. The sections cut it transversely only in the caudal portion; for the greater part of the plate they meet it tangentially or in a very oblique direction. For its form we must depend principally upon the reconstruction. From this it can be seen that there is a distinct primitive groove in its caudal fourth, measuring 0.25 mm.; otherwise the plate is smooth, with a slight tendency toward the formation of bilateral, low marginal ridges. There is, however, no evidence of a medullary groove. In the more trans-



verse sections, both at the caudal and rostral ends, the plate consists of stratified ectoderm about four cells thick and measuring 0.015 mm. midway between the median line and the lateral margin of the plate. Apparently this thickness is fairly uniform throughout the plate, although in many of the sections, owing to their obliquity, it would seem to be much thicker.

Laterally, along the margins of the plate, the ectoderm folds backward to become continuous with the amnion. At this point there is a transitional area consisting of ectoderm a single cell thick, which resembles the ectoderm of the embryonic plate more than that of the amnion. Since we have no knowledge as to exactly how much of the embryonic plate enters into the formation of the medullary plate, we can not as yet be sure of the destiny of this transitional area—whether to allot it to the amnion or to the integument of the body of the embryo. It would seem probable that it might be considered as an area of very active growth of the amnion. This would mean that the area of amnion formation is most active around its margins.

The primitive groove can be seen very clearly in the sections (figs. 12, 13, and 14, plates 2 and 3), and can be traced into the most caudal sections. Where one would expect to find the primitive node the sections become very oblique, so that it can not be outlined with great certainty. It is probably represented, however, in figure 14, plate 3. In this region the ectoderm fuses more or less completely with the endoderm, and lateral to the point of fusion can be seen a flattened area of mesoderm (fig. 13, plate 3). This mesodermal tissue is in the form of a reticula syncytium, which in most regions is closely attached to the ventral surface of the embryonic plate. It is somewhat more loosely attached to the endoderm by irregular trabeculae. In its more lateral areas it is slightly more condensed into a tissue from which are derived the somites and the more laterally situated unsegmented mesoderm. As to the presence of a head process there seems to be no evidence, although the oblique direction of the sections makes it impossible to rule it out with certainty. The most careful scrutiny, however, fails to reveal any sign of a head-process canal.

#### AMNION.

The amniotic cavity is much flattened and consists of scarcely more than a cleft. In this respect figures 15 and 16, plate 4, are very misleading, owing to the tangential direction of the sections. As has been seen, the amniotic ectoderm is directly continuous with the margins of the embryonic plate through a transitional zone. The amniotic ectoderm consists of a single layer of flattened cells which extends to the margin of the body-stalk, fitting tightly against its ventral border and conforming to its shape. This membranous layer of ectoderm is supported by a thin layer of parietal mesoblast, which is also somewhat membranous in character. On reaching the body-stalk the parietal mesoblast passes laterally, so as to partially inclose it. Made up of these two layers, the amnion forms a very thin membrane which lies in the narrow space between the chorionic membrane and the embryonic plate. Over the greater extent of its dorsal surface many loose strands of mesoderm partly attach it to the chorionic membrane. In figure 5 the cut edges of the amnion can be seen along the margins of the embryonic plate.

Careful search was made for an amniotic duct and it was found that the amniotic ectoderm, where it lies in contact with the body-stalk, shows at one point an active proliferation and the formation of a wedge which partially penetrates the body-stalk (fig. 14, plate 3). This may, perhaps, represent a tendency toward the formation of an amniotic duct.

#### BODY-STALK.

Loose strands of mesoderm are scattered at irregular intervals throughout the space between the amnion and chorionic membrane, whereas the center of the exocoelom is quite free from them. Within this area of looser mesenchyme is situated the more compactly arranged body-stalk, the form and structure of which can be seen by comparing figure 6, plate 1, and figures 13 and 14, plate 3. Lying at its center is the allantoic stalk, aside from which it consists entirely of a meshwork of mesoderm in which the process of angiogenesis can be seen to be under way. At the margins of the body-stalk the mesoderm is flattened into the mesothelium, which partially separates it from the exocoelom. The amniotic ectoderm bears a very intimate relation to the body-stalk, conforming closely to the shape of its anterior and ventral surface. It is at this point, as has been mentioned before, that the proliferating wedge of ectoderm penetrates into the substance of the body-stalk, representing the amniotic duct.

#### YOLK-SAC.

The yolk-sac is intact and forms a thin-walled, flattened vesicle, rather evenly distended, and measuring 1.5 by 1.4 by 0.9 mm. in its greatest diameters. It contains a moderate amount of finely granular coagulum, which is somewhat more abundant in the dorsal portion and around the margins. Over its dorsal pole the wall of the yolk-sac consists of two distinct layers—endoderm and visceral mesoblast—separated by a narrow cleft, which is bridged here and there by irregular trabeculae. In its ventral two-thirds the visceral mesoblast is intimately adherent to the endoderm, so that in many places it is difficult to make out more than one thin layer. The endoderm is quite uniformly made up of a thin, stretched out, single layer of membrane-like cells. The appearance of the uniformly thin wall of the yolk-sac is interrupted at intervals by small masses constituting the foci of blood-vessel formation. Sketches of these margins are shown on plate 5, and this process will presently be discussed.

Except at the line along which it fuses with the ectoderm, the endoderm shows very little difference from that in other regions. At the extreme caudal end of the embryonic plate, however, it consists of an area of taller and more cuboidal cells. This area evaginates at the center to penetrate into the substance of the abdominal stalk (fig. 9, plate 2) for a distance of 0.37 mm. as the allantoic duct. This is everywhere within the more compact body-stalk. About midway along its course the endoderm of the stalk seems to have broken off and retracted; in the interval, in some of the sections, the empty space previously occupied by it seems to be still present. The allantoic stalk, in some places along its course, shows a distinct lumen, while in other places this can not be recognized.

## ANGIOGENESIS.

Evidences of blood-vessel formation can be recognized in all parts of the chorion and the body-stalk and in certain areas of the yolk-sac. The mesoderm of the latter is very different in character from that of the body-stalk and chorion. To a lesser degree the mesoderm of the body-stalk and chorionic membrane differs from the mesodermic stroma of the villi; thus the picture of developing blood-vessels varies according to the region examined. We will therefore consider first the conditions existing in the chorion and body-stalk, which are closely allied, and then proceed to the vessel-formation of the yolk-sac, which has its own peculiar type of development.

As regards the chorionic membrane and villi, a careful survey shows very little difference in the number or degree of development of the blood-vessels in the different regions. In all parts of the chorion one can find the earliest types of vessels, consisting of simple protoplasmic, multinucleated strands, and the intervening stages between this and completed endothelial tubes. Selected stages of this process were sketched and are shown on plate 5, figures 17 to 22, which are arranged according to their apparent degree of differentiation. One gains the impression that the vessels are more numerous in the villi than in the chorionic membrane. This may, however, be due to the fact that the chorionic membrane is compressed into a compact sheet, consisting of 3 to 5 layers of flattened nuclei and their intermediate process-like strands. Owing to their arrangement these nuclei resemble endothelium, which makes the identification of endothelial formation uncertain. At the points where the mesoderm of the chorionic membrane evaginates to form the stroma of the villi, the trabeculae of the tissue are somewhat more loosely arranged and this makes it possible to identify the younger vessels with more certainty. The villi themselves offer a most favorable place for the study of angiogenesis. The arrangements here are extremely simple and the topography of the villi is such that one can select either cross or longitudinal sections at will. We have to take into consideration only a rather uniform stroma of the villus and its double-layered covering of epithelium. In the meshes of the stroma can be found the condensed strands which represent blood-vessels in their various stages of formation.

In the specimen we are describing, a great many of the villi do not show any sign as yet of blood-vessels. The villi devoid of blood-vessels are, as a rule, the smaller ones, and these are distributed evenly over all parts of the chorion. In some cases one can find a section showing a large villus with a mature main vessel, from which less mature strands can be seen extending into the terminal branches of the villus. These strands may be in the center of the villus or may extend obliquely so as to terminate close against the epithelium. The process by which blood-vessels are formed in the mesodermal tissue of the villi is initiated by the condensation of slender, cytoplasmic strands, along which are scattered irregularly placed and actively proliferating nuclei. We may speak of these as angioblastic strands which can be only incompletely resolved into separate cells. The next stage in the process consists in the differentiation of some of the component parts of

the angioblastic strand into endothelial cells, which can be distinguished by the shape of their nuclei and by their tendency to so arrange themselves as to form the contour of the strand. The cytoplasm of the other components of the strand undergoes liquefaction, either in the formation of large vacuoles or by resolution into a very fine mesh, which also disappears. The appearances in this respect probably represent the vacuolization phenomena demonstrated in the living chick by Professor Sabin, whose preparations I have had the privilege of examining and whose observations are reported elsewhere in this volume. In some cases we find a few round nuclei persisting, either adherent to the endothelial wall or suspended between the two endothelial walls by slender threads. These are doubtless to be regarded as future blood-cells. Whereas the spaces in the vacuolated strands are first seen to be incompletely closed off from the spaces of the surrounding stroma, further differentiation of the endothelium gradually completes their boundaries, thus forming completely closed tubes as established vessels. In other words, we are dealing with the formation of multinucleated strands, some of the elements of which become differentiated into endothelium, while the remainder is either completely liquefied or persists as blood-cells within the peripherally formed endothelial elements.

Figure 17 represents an angioblastic strand with its longitudinally arranged, elongated nuclei. In some places there is a slight indication of cleavage of the cytoplasm of such a strand, but this is always incomplete. Around its margins it is more or less continuous with the delicate trabeculae of the surrounding stroma. In figure 18 a similar strand is shown extending as a lateral process from the margin of a vessel that is farther advanced. Figure 19 illustrates the transition of a solid strand into an endothelial tube. Here the nuclei are in active proliferation. It can be plainly seen that the cells along the margin are elongating into typical endothelium, as regards both the nuclei and the adjacent cytoplasm. The cytoplasm of the central part of the strand shows enlarging vacuoles. At other points, such as the right-hand end of the figure, instead of showing a simple, large space, the cytoplasm becomes converted into a very degenerate mesh. As a result of these two processes there is a general liquefaction, or conversion into plasma, of the central portion of the angioblastic strand. Most of the nuclei seen in the strand in figure 19 have either divided or are about to divide. However, it is evident that some of them, together with their surrounding cytoplasm, must undergo degeneration.

Figure 20 shows a strand in which there is left only the differentiated endothelium. The incompletely closed lumen of this strand, as far as one can judge from the sections, still seems to communicate with the spaces of the surrounding stroma. In figure 21 we meet with a condition in which the endothelium forms a completely closed tube. A transverse section of a similar vessel is shown in figure 22. In figure 21 a few trabeculae still traverse the lumen connecting the opposite endothelial walls. There can also be seen within the lumen an occasional large, round, nucleated cell, showing a scant amount of cytoplasm, which apparently represents an embryonic blood-cell. This is the most mature type of blood-vessel encountered in the specimen. Up to this time there is apparently not a very active formation of blood-cells.

Owing to the uniform distribution of these different types of vessels throughout the chorion, there is strong evidence of the general differentiation of blood-vessels *in loco* rather than from a single focus restricted to any particular area.

In the region of the body-stalk one can recognize two areas of mesoderm: a more condensed area immediately surrounding the allantoic stalk, whose lateral margins are definitely inclosed by the formation of mesothelium and a much less compact area intervening between the former and the chorionic membrane. The more compact area is doubtless to be regarded as the forerunner of the permanent umbilical cord, whereas the looser area eventually is taken up by the exocœlom. In both of these regions blood-vessel formation can be seen taking place, and is in about the same degree of development as that noted in the chorionic membrane and villi. In some places throughout the looser areas of the body-stalk are small endothelial vesicles, which are completely detached from the adjoining exocœlom.

Blood-vessel formation can be detected over the greater part of the parietal mesoblast covering the amnion. In the body-stalk, as in the chorion, young blood-vessels are for the most part empty.

Angiogenesis in the yolk-sac presents a somewhat different picture from that seen in other parts of the ovum. In the first place it is circumscribed, being limited to the caudo-ventral half of the yolk-sac and is most marked at the extreme caudo-ventral pole; in the second place, the angiogenetic picture is quite different from that described as typical for the chorionic villi.

The wall of the yolk-sac consists of a thin, stretched-out, endodermic membrane, which is shown in figure 23, plate 5. This is covered in by the visceral mesoblast, shown below in the figure. In the more dorsal part of the yolk-sac the mesoblast is much thicker and more membranous and is separated from the endoderm by a distinct, narrow cleft. The cleavage between these two extends two-thirds of the distance from the dorsal pole to the equator and entirely encircles the embryonic area. Ventral to the cleavage rim a thin coating of visceral mesoblast fuses tightly with the endoderm, in some places being so thin that one can scarcely be sure that there is anything more than endoderm present. The simplicity of these regions of the wall of the yolk-sac constitutes very favorable conditions for the study of blood-vessel formation.

Sketches were made of selected areas of the wall, showing blood-vessels in their different stages. These are arranged in figures 23 to 28, plate 5, in their approximate order of development. All of the figures are so arranged that the endoderm is above and the mesoderm below. In figure 23 is shown a small, isolated clump of proliferating mesoblast cells in an area where the endoderm is only scantily covered. From such an angioblastic node we can find all stages of transition up to completed endothelial tubes. In figure 24 is another clump, slightly larger, but otherwise of much the same character. From their appearance one could not be sure that such clumps were destined to form blood-vessels. Since, however, there is at this time no other process taking place, we may assume that these are earlier stages of the condition met with in figure 25. In this figure there is shown a characteristic, compact, multinuclear plate, in which the cellular boundaries can be only partially

made out. In such plates one can very early recognize that certain cells at the periphery are becoming elongated and flattened into endothelial cells. These shape themselves so as to compactly inclose the more centrally placed cells. It is also characteristic of these angioblastic plates that the cytoplasm of the more centrally placed cells undergoes liquefaction similar to that in the long strands in the chorionic villi. The liquefaction of such cells seems to vary, since it is found in some of the smaller angioblastic plates and is absent in some of the larger ones.

In figure 26 is shown a larger angioblastic mass with less vacuolization and liquefaction of the cells than in the preceding figure. However, endothelial cells differentiating around the contour of the mass can be distinctly recognized. The component cells of the mass show evidences of very active proliferation; they are either in mitosis or in pairs of small, recently divided nuclei. In figure 27 the condition is more advanced and a considerable amount of liquefaction may be seen among the more centrally placed cells. In addition to large vacuoles, one finds in such a mass that many of the nuclei are becoming very large and pale, apparently preliminary to their complete disappearance. Although the endothelium can be apparently recognized, it has not yet completely closed off the area from the exocoelom, and even less so from the endoderm. In a few cases completely formed endothelial tubes are found on the yolk-sac, as shown in figure 28. These may contain one or more cells with large, round nuclei, but never so many as are present in the angioblastic masses seen in figure 27. We must conclude, therefore, that there is in these cases a considerable conversion of the cellular mass into clear plasma, leaving relatively few complete cells, none which as yet show any evidence of the presence of hemoglobin.

#### COMPARISON OF THE MATEER EMBRYO WITH OTHER YOUNG HUMAN EMBRYOS.

As an aid in placing our specimen in its proper relative position in the series of embryos that have been described in the literature, the more important of these will be briefly reviewed. They will be taken up in the order of their apparent degree of development, which will be determined by the consideration of the morphology as well as by the actual size of the embryo and the chorion. The manner of handling and the amount of shrinkage and folding affect the size greatly, particularly as regards the dimensions of the chorion. The disproportion between size and development is even greater where pathological elements have entered. On the other hand, in young stages up to the time of the appearance of the primitive groove, the size of the chorion, owing to its rapid growth as compared with that of the embryo, appears to be a consistent index of the development of the ovum. In older specimens it is necessary to take into account also the morphology of embryo and chorion.

For the most part the literature dealing with young embryos relates to the histological character of the implantation area and to the interaction between the trophoblastic shell and the uterine mucosa; the structure and form of the embryo, with which we are especially concerned, are given with much less detail. This is due in part to the inadequacy of the material, the chorion and trophoblast being

usually in a better state of preservation than the embryo. There will be considered here only presumably normal embryos in which the somites have not yet made their appearance. These will be taken up in three groups: (1) Those in which the primitive groove is not yet formed; (2) those in which the primitive groove is present; and (3) those having, in addition to the primitive groove, a neurenteric canal and medullary folds. A list of these, with their measurements, is given in table 1.

TABLE 1.—*Dimensions of human ova of the presomite period.*

Author.	Chorion.		Amniotic cavity.	Yolk-sac.	Embryonic shield.
	External diameters.	Internal diameters.			
GROUP 1.					
Miller, 1913.....	0.83	0.44	.....	.....	0.095*
Bryce-Teacher, 1908..	1.95 by 1.1 by 0.95	.77 by 0.63 by 0.52	.....	.....	.15*
Linzenmeier, 1914....	.....	.75 by .61 by .52	0.1 by 0.09	.....	.21 by 1.05*
Peters, 1899.....	2.4 by 1.8	1.6 by .9 by .8	.....	.....	.19*
Fetzer, 1910.....	2.2 by 1.8†	1.6 by .9	.22	0.2	.23
Heine-Hofbauer, 1911.	.....	2.3 by .98	.....	.....	.....
Jung, 1908.....	.....	2.5 by 2.2 by 1.0	.....	.....	.....
Merttens, 1894.....	4.0 by 3.0	3.0 by 2.0	.....	.....	.....
Strahl-Beneke, 1910..	.....	3.8 by 2.2 by 1.2	.....	.....	.75 by 0.3
GROUP 2.					
v. Spee ("v. H."), 1896	6.0 by 4.5	4.0	0.76 by 0.76	1.05 by 1.08	0.37 by 0.23
Debeyre, 1912.....	12.0 by 7.5 by 4.0	4.7 by 2.6†	.....	.9 by 0.84 by 0.55	.85 by .56
Mateer (Embryo No. 1399).....	9.0 by 8.0 by 3.5	6.1 by 5.6 by 2.5	.92 by .78	1.5 by 1.4 by .9	.92 by .78
van Heukelom, 1898..	.....	7.5 by .1	.....	.....	.3
Giacomini, 1898.....	8.5	.....	.5	1.0 by .4	.....
GROUP 3.					
Keibel (Bayer), 1890..	8.5 by 7.7 by 6.0	.....	.....	1.0	1.0
Ingalls, 1918.....	9.1 by 8.2 by 6.5	8.0 by 7.0 by 5.0	.....	2.5 by 2.0	2.0 by 0.7
Grosser, 1913.....	10.0 by 8.0	8.0 by 6.0	1.07	1.6 by 1.2	.83 by 0.5
Strahl, 1916.....	10.0	.....	.....	.....	.7
Frassi, 1907.....	13.0 oy 5.0	9.4 by 3.2	.....	1.9 by 0.9	1.17 by 0.6
Eternod (Vuill), 1899.	10.0 by 8.2 by 6.0	9.0 by 7.2 by 5.0	.....	.....	1.3
v. Spee (Gle), 1889, 1896.	.....	10.0 by 8.5 by 6.5	.....	.....	1.54

\* Embryonic mass.

† Measurement taken from author's illustration.

## GROUP 1. EMBRYOS BEFORE THE FORMATION OF A PRIMITIVE GROOVE.

The youngest stage in the development of the human embryo that has thus far been observed is represented by a blastocyst already embedded in the uterine mucosa, but devoid as yet of villi. Two such specimens have been described, one by Miller (1913), the other by Bryce and Teacher (1908). The one described by Miller is smaller and in it the embryonic rudiment consists of a solid mass of cells, whereas in the Bryce-Teacher specimen an amnio-embryonic vesicle can be recognized. Certainly the Miller specimen must be regarded as normal, and although the Bryce-Teacher specimen shows some evidence of degeneration it also should be provisionally regarded as normal. When more specimens of about the same age are available for comparison it is quite possible that the evidence may prove that the latter is not normal.

The specimen described by Miller was found in sections of material obtained at curettage. It was not a complete series, only five sections showing the ovum. Fortunately, however, three of these passed through the embryo, which is repre-



sented by a solid cell mass 0.095 by 0.072 mm., undergoing cleft formation preliminary to the development of the amniotic cavity. The embryo is surrounded by a trophoblastic shell with an external diameter of 0.83 mm. and an internal diameter of 0.44 mm. The trophoblast is partially differentiated into cytotrophoblast (Langhans layer) and plasmoditrophoblast (syncytium), the two partially merging into one another. Peripherally, the plasmoditrophoblast forms irregular loops inclosing large blood lacunæ, about 12 in a single section, and gives the appearance of eroding and engulfing the surrounding capillaries. There is no evidence of villi. The interval between the trophoblastic ectoderm and the embryo is almost entirely filled with a fine, granular deposit, through which pass simple fibroblastic strands. These are arranged in a layer which lines the trophoblast, and are especially numerous on one side of the embryo, between it and the trophoblastic ectoderm, apparently the area of the body-stalk.

The Bryce-Teacher ovum, both from its size and in its development, is to be regarded as slightly older than the Miller specimen. It differs from the latter in the following particulars: The trophoblast shell is surrounded by a necrotic decidual area. The distinction between the cytotrophoblast and plasmoditrophoblast is more definite, and the latter forms a more complicated network. There is no evidence of any arrangement of the mesoderm into parietal and visceral lamellæ—it still fills the blastocyst as a delicate tissue in the fine meshes of which the embryo is suspended. The latter consists of two detached vesicles; the larger (diameter 0.168 mm.) is the amnio-embryonic vesicle with a wall of cubical cells; in the smaller the yolk-sac (diameter 0.042 mm.) the cells are flattened. That these two vesicles are completely detached and not in proper relation to each other would tend to indicate that the specimen is not entirely normal.

The beginning formation of villi can be seen in the ova described respectively by Linzenmeier (1914) and by Peters (1899). Of these, the former is smaller and probably younger; it is, however, distinctly older than the Bryce-Teacher specimen, in respect to both the chorion and the embryo. The mesoblast is divided into parietal and visceral layers, with a well-defined exocœlomic cavity. The parietal mesoblast forms a continuous layer within the trophoblastic ectoderm, completing the formation of the chorionic membrane. Short processes from its mesoblastic layer project outward into the trophoblastic shell, producing the first villi; seven or eight of these may be seen in a single section. The mesoblastic layer of the chorionic membrane is continuous with the visceral layer of mesoblast surrounding the embryo, at the seat of the future body-stalk. The embryo consists of two closely opposed vesicles; the larger (greatest diameter 0.105 mm.) is clearly differentiated into amniotic ectoderm and a thickened ectodermal embryonic plate; the smaller constitutes the yolk-sac and is about half as large as the amniotic vesicle. It is partially separated from the former by a layer of mesoderm. Whether an allantois is as yet present could not be definitely determined from the author's description. From the illustration the wall of the yolk-vesicle seems to consist of a very thin membrane.



In the well-known Peters ovum, which is much more completely described, the conditions are closely similar to those in the ovum just referred to. In it the villi are more numerous and distinct and the chorionic vesicle is larger. In form and position this embryo is almost identical with the Linzenmeier specimen, the amnion lying in contact with the mesoblastic layer of the chorionic membrane. Both possess closure-caps (*Gewebspilz*) consisting of partially organized fibrin and blood, into which the trophoblast sends branches.

As will be seen, the embryos of the first group, before the formation of the primitive groove, fall naturally into three substages: (1) those without villi, (2) those possessing primitive villi, and (3) those in which the villi have already begun to branch. The first two have just been considered, and we may now take up those with branched villi. There are three ova, described respectively by Fetzner (1910), Heine-Hofbauer (1911), and Jung (1908), which are a little larger and a little further differentiated than that of Peters. The three are very much alike in form and about the same size. They have numerous and well-defined chorionic villi, which show a beginning tendency to branch. In all of the specimens the yolk-sac is smaller than the amniotic cavity and its wall consists of a single layer of flat endodermal cells. The embryonic plate presents an oval surface and in section consists of two or three layers of high cylindrical cells, whereas the amniotic ectoderm, in sharp contrast, consists of only a single layer of flat cells.

The Herzog (1909) ovum is about the same size as those just mentioned. Its chorionic wall, however, is much folded, so that originally it was probably considerably larger. It contains a tubular structure in the body-stalk, obviously torn from the embryo in the process of preservation and thus is completely detached. This epithelial tube was at first interpreted as an allantois, but, as subsequently determined by Professor F. T. Lewis (1917), it is clearly an amniotic duct, no allantois being present in this embryo. Apparently the yolk-sac is larger than the amniotic cavity (largest diameter of yolk-sac 0.3 mm., amniotic vesicle 0.16 mm.).

The Strahl-Beneke (1910) ovum, which the authors have published in a splendid monograph, would fall between our first and second groups. The histological appearance of its chorion differs only slightly from those just referred to. In form it is more elongated, presenting a spindle shape that is not repeated in any other ovum and probably should not be regarded as typical. The embryo shows certain new features as evidence of a more advanced stage of development. In the body-stalk there is a solid epithelial strand connecting the tip of the amniotic sac with the chorionic ectoderm and apparently representing an amniotic duct. On the ventral side of the yolk-sac are thickened, solid mesodermal strands which are possibly predecessors of blood-vessels. These are not to be confused with the ring-like arrangement of mesodermal cells which simulates vessels and may be seen in all mesodermal parts of the ovum. In figure 32 (Strahl-Beneke) there is seen a clear area in the ectodermal plate which suggests the transition of the cells into a *canalis neurentericus*, although a distinct canal is not present. The authors also speak of a head-process, including under that term the free mesodermal cells which, in the middle line, beneath the embryonal area, lie between the ectoderm and endoderm,

as seen in their figures 41 and 42. More certain than either of the last two phenomena is the beginning appearance of the primitive streak. In their figures 25 to 34 it can be seen that the ectoderm of the caudal end of the embryonic plate fuses with the mesoderm, producing the appearance of a primitive streak, although there is no distinct groove. In this ovum, as in the Herzog specimen, the yolk-vesicle is distinctly larger than the amniotic vesicle, differing in this respect from all those previously mentioned. The Herzog and Strahl-Beneke specimens are on the border-land between groups 1 and 2, and, except for the absence of a primitive groove, could be grouped with the "v. H" ovum of Graf v. Spee.

Together with the Strahl-Beneke specimen should be mentioned the Merttens (1894) ovum, which is about the same size. Unfortunately, the sections are incomplete, being fragments from curettage, so that it is not possible to determine much regarding the structure of the embryo. The chorion, however, is well preserved and acquires great importance because of the valuable clinical history that accompanies the specimen. Another of about the same age as the Strahl-Beneke ovum is No. 763, of the Carnegie Collection, which has been mentioned by Mall (1915, p. 22), and a photograph of which is shown in our figure 7, plate 1. The specimen was found in curettage material and the series is therefore incomplete. The tissue, however, is in an excellent state of preservation and the structure of the chorionic membrane and villi is very well shown, as can be seen in the photograph. The internal diameters of the chorion in the largest section are 2.5 by 1.2 mm., and the average length of the villi is 0.5 mm.

#### GROUP 2. EMBRYOS IN WHICH THE PRIMITIVE GROOVE IS PRESENT.

The embryos belonging in this group have a primitive groove but no medullary groove or neurenteric canal. Five such specimens are referred to in the literature, and in this group the Mateer embryo must be placed. They are all much alike in size and form and evidently there is very little difference in their degree of development. As a group they are of a size intermediate between groups 1 and 3.

The well-known "v. H" ovum of Graf v. Spee (1896) is perhaps a little less developed than the others. In the relatively small size and spherical form of its amniotic vesicle it resembles some of the older specimens of group 1 and its embryonic shield is quite like that of the Jung specimen except for the presence of a primitive groove. It has, however, a larger chorion (average internal diameter 4 mm.) with freely branching villi. The yolk-sac is much larger than the amniotic cavity, and there is a well-developed allantoic duct extending from it into the body-stalk. On its ventral pole are found numerous blood-islands. In all these respects it conforms to the other specimens of group 2.

Lewis (1912) has pictured and briefly described the Minot embryo (Harvard series, No. 825), and I am informed that a more complete description of it is now in course of preparation. This embryo resembles very closely the "v. H" embryo of v. Spee, both in size and in form. It differs in that the amniotic vesicle is more flattened and the embryonic shield correspondingly larger. Whether the spherical form of the amniotic vesicle normally precedes the flattened form usually met with

in slightly larger specimens, remains to be determined by a comparison of more material than is available at present. It is quite probable that the form of the vesicle is dependent to a great extent upon such factors as the handling and preservation of the tissues and the technique of embedding. In the Minot specimen there is a distinct primitive knot where the ectoderm and endoderm are definitely blended. Numerous blood-vessels are present in the wall of the yolk-sac and also in the body-stalk and the chorion.

In addition to the two just mentioned, there are three other specimens closely resembling our own. These have been described respectively by Debeyre (1912), van Heukelom (1898), and Giacomini (1898). Of these, the Debeyre ovum is in the best state of preservation. In it the embryonic shield is in the form of an elliptical and dorsally convex plate, consisting (according to the author's description) of a single layer of cylindrical epithelial cells showing numerous karyokinetic figures. In consisting of a single layer of cells it differs from the embryonic shield in the other specimens mentioned, in all of which it was described as stratified. Debeyre explains the appearance of stratification as due to the distribution of nuclei at different levels. There is a well-marked primitive streak, along which exists a close union between the ectoderm and endoderm, and a primitive groove 0.54 mm. long. At the caudal end of this there is a cloacal membrane where the ectoderm and endoderm again join. No trace of a neural groove or neurenteric canal could be found. The amnion incloses a flattened space conforming to the shape of the embryo. There is no amniotic canal present. An allantois 0.4 mm. long is present and has a distinct lumen, but no terminal dilatation. The yolk-sac is lined with endoderm, which has the appearance of a protoplasmic syncytium sown with nuclei; the contours of the cells, for the most part, can not be made out. There are no epithelial buds or glandular diverticula. The supporting mesoderm is extremely variable and at places is entirely lacking. At the ventral pole of the yolk-sac, apparently arising from the mesoderm, are numerous blood-islands in the following forms: (1) Full and consisting of many layers of cells arranged concentrically; (2) an opaque mass of amorphous substance sown irregularly with nuclei of uniform size; (3) cellular elements arranged around a central cavity; (4) uniform cells surrounding a cavity which may or may not have partitions and which contain differentiated elements, some having large nuclei with little protoplasm; (5) irregular strands of closely packed mitotic cells. Blood-islands are also present in the body-stalk, but none are to be seen in the region of the embryonic plate.

In the van Heukelom specimen the chorion is torn and collapsed and the tissues are in a rather poor state of preservation. From its general form and size, however, one can see that it closely resembles the other specimens of this group. In the brief anatomical description mention is made of the presence of the primitive groove, allantois, and blood-islands in the walls of the yolk-sac, and what were possibly blood-vessels in the chorionic membrane.

The Giacomini specimen is in form very much like the "v. H" ovum of v. Spee, but from the figures and description it is apparent that it had undergone marked maceration. Owing to mechanical injury the yolk-sac is partially detached from

the amniotic vesicle. The primitive groove and allantois are present. In the wall of the yolk-sac are cell groups that seem to be blood-islands, but no definite vessels were found in the body-stalk or chorionic membrane.

The two ova described respectively by Reichert (1873) and Rossi Doria (1905), both of which are frequently referred to and which are about the same size as those in this group, can not, however, be used for this comparison, as no description of the embryo is given by either of the authors.

A summary of the features occurring in all of the embryos of this group would include the presence of a primitive groove and an allantois, with evidences of blood-vessel formation in the wall of the yolk-sac and (in most of them) also in the body-stalk and chorionic membrane. Furthermore, in all of them the yolk-sac is considerably larger than the amniotic cavity. The chorion is covered with freely branching villi and its ectoderm is clearly differentiated into two layers. The mesoderm lining the chorion forms a well-defined supporting membrane, from which processes extend as the cores of the villi. The internal diameter of the chorion of such an ovum is from 4 to 6 mm.

All of these characteristics are present in the Mateer specimen. In addition, the latter, as we have seen, also shows evidences of blood-vessel formation in the villi, which was not reported in the others. It may be that the Mateer ovum is more advanced in development and is thus on the border-land between groups 2 and 3. On the other hand, it is apparently in a better state of preservation than the others of this group, a factor of great importance in the recognition of the early stages of vasculogenesis. In the other specimens the process may have been obscured by the poor preservation of the tissues.

### GROUP 3. EMBRYOS HAVING MEDULLARY FOLDS AND A NEURENTERIC CANAL.

The first three specimens to be mentioned in this group are those described respectively by Grosser (1913), Strahl (1916), and Ingalls (1918). These resemble each other very closely, both in form and in size, and all are in a state of good preservation. In each the greatest external diameter of the chorion is about 10 mm., the greatest internal diameter about 8 mm., and the yolk-sac nearly 2 mm. in its greatest diameter. The only marked difference in size is found in the embryonic shield, which in the Grosser and Strahl specimens is slightly less than 1 mm. long, while in the Ingalls specimen it is 2 mm. long. In all of these embryos there is a distinct head-process with its contained canal,<sup>1</sup> together with a completion-plate. One can also now speak of medullary folds, and at the caudal end of the primitive groove the ectoderm and endoderm unite in the formation of a well-defined cloacal membrane. Thus, both in the differentiation of these particular features and in

<sup>1</sup> A complete description of this canal is given by Ingalls, who designates it as the *archenteric canal*, holding, contrary to Hubrecht and Keibel, that in the formation of the head-process all the essentials of gastrulation are represented, and that its lumen is in reality an archenteric canal. Furthermore, according to him, the frequently used term *chordal*, or *notochordal canal* is inadequate, since only a part of the wall enters into the formation of the chorda. Similarly, an objection might be raised to the use of the term *neurenteric canal* as a designation for the canal in its earlier form, as in the strict sense this can refer only to the caudal portion of the canal of the head-process—the short, sharply defined canal connecting the caudal end of the floor of the medullary groove with the gut, as is best seen in the older specimens of this group.

their size, these ova represent a stage definitely in advance of that seen in the specimens of group 2.

In the Grosser specimen an amniotic duct is described as connecting the amniotic ectoderm with the ectoderm of the chorionic membrane. A similar structure has been seen in the much younger Strahl-Bencke (1910) embryo. In the Strahl (1916) specimen there exists in the body-stalk a small remnant of what seems to be an amniotic duct in process of disappearing. In the Ingalls specimen, however, neither the presence of the amniotic duct nor evidence of its recent disappearance could be made out. There is a short amniotic diverticulum extending from the amniotic cavity towards the allantois, which is regarded by Ingalls (p. 21) as comparable to the *canalis amnioallantoideus* connecting these cavities in certain reptiles.

The Bayer ovum described by Keibel (1890), judging from its size and general form, would represent about the same stage of development as those just mentioned. Unfortunately, the preservation is not good, and much of the detailed structure of the specimen is lost. The embryonic shield shows a well-developed primitive streak, but whether a head-process and a neurenteric canal are present could not be verified.

The Frassi (1907) specimen, which was studied in Professor Keibel's laboratory, is a little larger than those thus far mentioned in this group. It possesses a medullary groove bordered by evidences of medullary folds. A *canalis neurentericus* connects the caudal end of the floor of the groove with the amniotic cavity. A definite cloacal membrane is present. The amniotic and vitelline cavities are distended and thin-walled; in structure they are essentially the same as those found in all of the specimens of groups 2 and 3. The same is true of the allantois, the chorion and the chorionic villi. These features do not offer definite criteria for determining the relative degree of development of the specimens of these two groups, nor can the observations on blood-vessel formation be safely used for this purpose, for the reason that the reported presence or absence of blood-islands and blood-vessels in the various parts depends largely upon the state of preservation of the specimen, and also upon the observer's familiarity with the early form of these structures. The Frassi specimen showed no evidence of an amniotic duct.

The last two specimens that should be included in this group are the frequently referred to Vuillet specimen of Eternod (1899) and the Glaevecke specimen of v. Spee (1899). One should, perhaps, add to these the Triepel (1916) specimen, which seems to be in about the same stage of development, although in not quite as good condition. The v. Spee ovum is slightly more advanced, both in form and size, than that described by Eternod; otherwise there is a very close resemblance between the two. Each has a distinct medullary groove, bordered by broad medullary folds corresponding to the head-region. A well-defined chordal plate lies along the ventral surface of the medullary groove and terminates caudally at a large, distinct neurenteric canal, where its cells are continuous with those of the ectodermal cells of the medullary plate. Caudal to the neurenteric canal, in the region of the primitive streak, the axis of the embryo bends sharply ventralward to terminate in the

body-stalk. Just before this point of termination the ectoderm and endoderm unite in the formation of a cloacal membrane. The dorsal wall of the yolk-cavity shows a beginning constriction, which marks off the gut-area. In this respect the v. Spee is slightly more advanced than the Eternod specimen; in it a distinct pouch, corresponding to the fore-gut, is marked off. As regards blood-vessels, it also shows an advance over any of the embryos thus far referred to.

Throughout groups 2 and 3 we find evidences of blood-vessel formation in the wall of the yolk-sac, in the body-stalk, chorionic membrane and chorionic villi; here, however, for the first time, developing blood-vessels are found in the body of the embryo. Distinct strands, which are to enter into the formation of cardiac endothelium in the v. Spee specimen, are pictured by Evans (1912). Eternod describes in his own specimen a complete blood-circulation. It includes a heart with three or four aortic arches and paired aorta extending back to the body-stalk, where they break up into branches that are distributed to the villi. The blood is returned by two large veins that unite to form a vena umbilicalis impar; this in turn bifurcates into two chorio-placental veins which extend along the junction of the embryo and yolk-sac, to unite in front at the venous end of the heart. From Eternod's description, these vessels are only partially permeable. For the greater part they are in a mesodermal condition which (he says) renders them difficult to trace. It is therefore quite possible that the actual differentiation of the blood-vessels is not so far advanced nor the circulation so complete as one would be led to believe from the author's description. Evans (p. 592) regards it as certain that the structures described by Eternod as aortic arches are not such, but only the components of a vascular plexus. We can, however, safely assume that angiogenesis is to be recognized in the body of the embryo at this time.

In a summary of the embryos of this group, as compared with those of group 2, there is to be included the formation of the medullary groove and medullary folds, the formation of the head-process and its contained canal, the differentiation of the neurenteric canal and the chordal plate, the formation of the cloacal membrane, the beginning constriction of the gut-portion from the remainder of the yolk-sac, and finally the evidences of angiogenesis within the body of the embryo.

#### PROBABLE AGE OF THE MATEER EMBRYO.

The sequence of morphological events that mark the developmental period from the earliest-known form up to the appearance of the first somites is now fairly well established and, as can be seen from the preceding review, the embryos therein referred to may be arranged in a consecutive series of clearly defined stages. These may be briefly summarized as follows:

In the first stage the chorionic villi have not yet appeared and the embryonic rudiment consists of two simple vesicles or of a solid mass of cells in the process of forming these vesicles. In the second stage the villi have made their appearance but are still of a primitive character. In the embryo a mesoblast can be recognized, and is separated into parietal and visceral layers forming an exocoelomic cavity, and the ectoderm can be resolved into the amniotic ectoderm and that forming

the embryonic plate. In the third stage the villi are branched and the size of the chorion has increased relative to that of the embryo. The first three stages as a group include all those specimens in which the primitive groove has not yet formed. The fourth stage is marked by the presence of a primitive groove; there is also a well-developed allantoic duct, the yolk-sac has become larger than the amniotic vesicle, and the first steps in the formation of blood-vessels can be recognized in the wall of the yolk-sac and in the chorion. In the fifth stage, in addition to a primitive groove, there is a distinct head-process with its contained canal, and also a completion-plate. Medullary folds can be recognized, and at the caudal end of the primitive groove the ectoderm and endoderm unite in the formation of a cloacal membrane. In the sixth stage there is further differentiation of the neurenteric canal, formation of the chordal plate, beginning constriction of the gut-endoderm from the remainder of the yolk-sac, and finally evidences of angiogenesis within the body of the embryo. The fifth and sixth stages make up our third group of specimens of the presomite period.

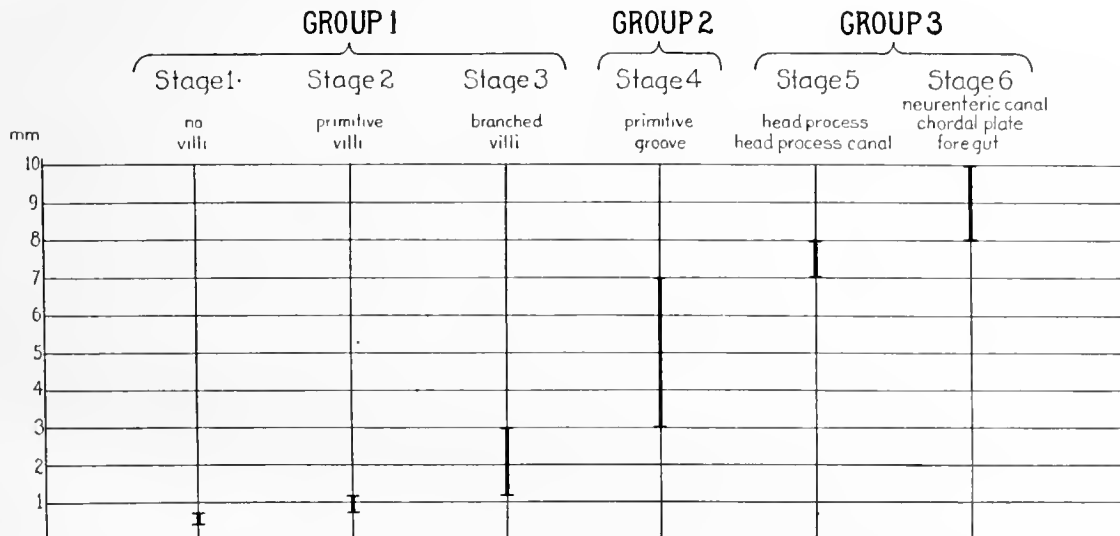


CHART 1.—Size of the human ovum during the presomite period, showing range of the greatest internal diameter of the chorion (in millimeters) of all the human embryos thus far published, from the youngest-known stage up to the appearance of the somites.

In addition to these morphological changes there is a corresponding change in the size of the different ova. This is shown in chart 1, in which the size of the ovum is represented by its most easily determined dimension—the largest diameter of the chorionic cavity. The range of this dimension, as can be seen in table 1 and in chart 1, falls within limits that correspond consistently to the different stages of development. In the only specimen in which there is a departure from this dimensional curve (Strahl-Beneke ovum) the discrepancy is due to an extreme elongation of the chorionic sac.

From such morphological and dimensional criteria it is possible to determine the relative development of any two specimens with a considerable degree of accuracy, which is of the greatest importance in arriving at the true age of an



embryo. Whereas the clinical data for any individual specimen may be incomplete, when used conjointly with data from similar specimens it may prove of decisive value. In the case of two specimens of the same developmental period, if we know, for instance, that one can not be younger than 15 days and the other not older than 18 days, we have then established the probable age-limits for all the specimens of that group. It may be explained here that by the term *age* is meant the *fertilization age*; that is, the time elapsing from the fertilization of the ovum to the cessation of development. This term was introduced by Mall (1918), who distinguishes it from the *copulation age*, which he shows to be approximately 24 hours longer.

Decisive age-data of the character referred to are available in connection with the six specimens listed in chart 2, and we are thus enabled to place limits within which the age of the specimens belonging to our three main groups must fall. In

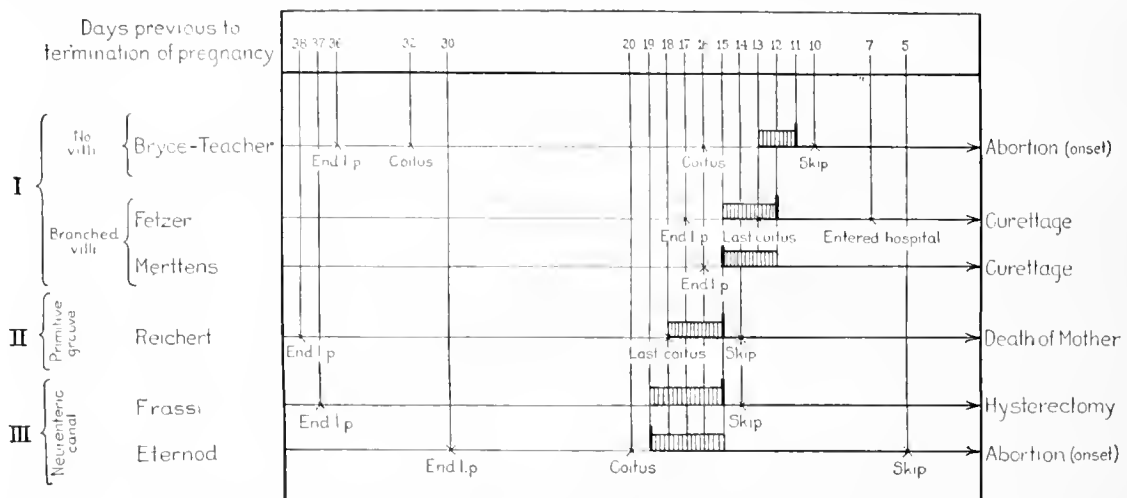


CHART 2.—The possible duration of pregnancy in 6 cases having crucial clinical data. Allowing 24 hours each for the interruption of the menstrual period and the completion of the abortion, the Bryce-Teacher specimen could not have been older than 11 days. The Fetzer specimen is probably not younger than 12 days, and the Merttens specimen not older than 15 days; being in the same stage of development, these two furnish the range of possible age for all specimens of similar development. In like manner the Frassi and Eternod specimens furnish the range of possible age for group 3—i. e., 15 to 19 days.

the Bryce-Teacher specimen the patient missed her period 10 days prior to the onset of the abortion. If we allow an additional day as the minimum time necessary for the interruption of the expected period, the total minimum age would be 11 days. In the Fetzer specimen the last coitus, according to information given by the patient, was at least 6 days before entering the hospital, and 13 days before the removal of the specimen by curettage. If 24 hours are allowed for fertilization to take place the result is a minimum age of 12 days. In the Merttens specimen the last menstrual period began 21 days prior to the curettement and lasted 5 days. Allowing one day for fertilization, there are left 15 days as the maximum age. Since the Merttens and Fetzer specimens are of about the same stage of development we are justified in concluding that they can not be older than 15 days or younger than 12 days, as indicated in chart 2. The Bryce-Teacher specimen, being



in a distinctly younger stage of development than either of these, must be considered as correspondingly younger, though it can not be younger than 11 days.

The morphological description of the Reichert (1873) specimen is in many respects inadequate. From its dimensions, however, it must fall in our second group, the embryos of which have a primitive groove but no head-process canal. The patient in this case died of suicidal poisoning 14 days subsequent to the omission of her menstrual period. If an additional day is allowed for the interruption of her period it makes a minimum age of 15 days for this group. There are no clinical data from the other specimens of this group which would make possible a conclusion as to the maximum age for this stage of development. It must lie somewhere between the maximum age of the preceding and that of the succeeding group—that is, between 15 and 19 days.

In the third group data as to maximum and minimum age are furnished by the Frassi (1907) and Eternod (1898) ova. In the Frassi case the patient reported that her last menstrual period began two weeks before the hysterectomy, but upon subsequent careful questioning it was learned that she had missed her period 14 days prior to the operation. Allowing an additional day for the interruption of the period, this leaves a minimum age of 15 days. In the Eternod specimen the abortion of a well-preserved, normal ovum followed 21 days after a single coitus. Assuming one day for the process of fertilization, there are left 19 days as the maximum age. From the foregoing it is apparent that the period of development from the time of the formation of the ectodermic vesicle (Bryce and Teacher) up to the time just preceding the appearance of somites (Eternod) does not cover more than 8 days (eleventh to nineteenth day).

While the data in each of these selected cases tend to substantiate that of the others, some of them nevertheless are not entirely above question. In the case of the Bryce-Teacher specimen one might raise the point that, although at least 11 days elapsed from fertilization to the beginning of the abortion, we can not be sure that the embryo continued in its development up to that time. It may have died a few days previous, thus making a minimum age of less than 11 days. In the Fetzner case we are allowing 24 hours for fertilization following the last coitus, which is probably adequate, although we know that spermatozoa may retain their vitality much longer than this. In the Merttens specimen there can be no doubt as to the accuracy of 15 days as the maximum age. The same is true of the Reichert and Frassi specimens, in which 15 days is certainly their minimum age. In the case of the Eternod specimen we must depend on the reliability of the patient's statements and at the same time assume that 24 hours is sufficient for fertilization. That we are safe, however, in accepting the confirmatory trend of these data is supported by the recent observations of Huber on the rat, in which it was possible to obtain timed specimens.

In addition to the above 6 specimens in which decisive data as to maximum and minimum age are present, there are 11 other specimens of the presomite period, including our own, which have available clinical records. These are arranged in the order of their development in chart 3, and the probable duration of pregnancy in the individ-

ual specimens is arrived at by their adjustment to the maximum- and minimum-age data, as indicated by the heavy brackets. The ages fall within these limits and thus form a curve which would correspond to the gradual increase in size and the differ-

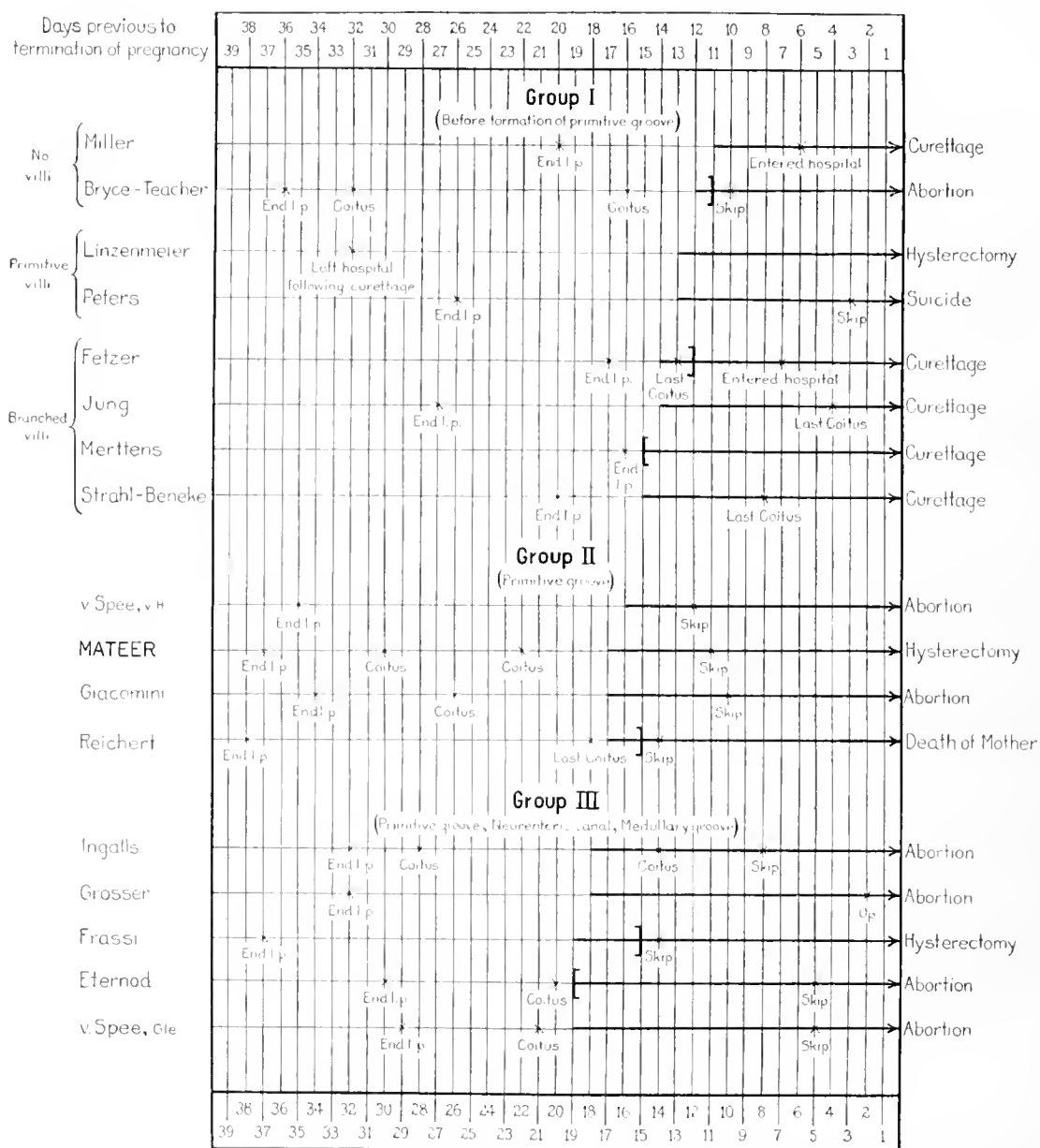


CHART 3.—In this chart are shown the clinical data of the human embryos of the presomite period, arranged from above downward in the order of their development. Brackets are introduced in those cases having crucial age-data, and the ages for the different stages of development must fall within the bracketed periods. In this way the probable age of each specimen was determined, and this is indicated by heavy horizontal lines.

entiation of the individual specimens. The essential clinical facts as far as reported are indicated in the chart and need not be detailed here. It should be said, however, that in the cases terminating in abortion 24 hours are allowed as an average dura-

tion of that process; that is, development is regarded as arrested 24 hours before the completion of the abortion.

The Mateer specimen is probably not younger than the Reichert specimen, which in turn can not be younger than 15 days. On the other hand, it is distinctly younger than the Eternod specimen, which is not older than 19 days. If the Ingalls and Grosser specimens, which are also younger than the Eternod specimen, are placed at 18 days, then the Reichert, Giacomini, and Mateer specimens, being still younger, would fall back on the 17th day, which is probably very close to their actual age.

On examining chart 3 it will be found that, if the age-deductions are correct, pregnancy in 16 of the cases listed began respectively 1, 1, 3, 4, 5, 9, 10, 11, 13, 13, 14, 14, 17, 18, 19, and 20 days following the end of the last menstrual period.<sup>1</sup> In no case did it begin during the period or in the 4 days immediately preceding it. It would appear that conception takes place most frequently (25 per cent of the above cases) at about the 13th or 14th day following the cessation of the menstrual period. In nearly one-half of the cases it occurred at the end of the second and during the third week, which has previously been regarded as a comparatively sterile period.

#### TWIN EMBRYO.

Before concluding I wish to call particular attention to the two small vesicles which are to be found in the region of the body-stalk, mention of which was made under the serial description of sections (secs. 68 to 79). The opinion was there expressed that these structures constitute respectively the amniotic vesicle and yolk-vesicle of another embryo. Inasmuch as an opportunity is thus afforded to observe twin-formation at an extremely early stage, this specimen has an important bearing upon the problem of that process.

As we have seen, intervening between the amnion and the chorionic membrane there is an area which is partially filled with parietal mesoblast. Caudally, this area surrounds the body-stalk proper, which can be distinguished by its more compact structure. Around the base of the body-stalk the parietal mesoblast walls itself in from the exocoelomic cavity by a membranous arrangement of its superficial cells in the form of a mesothelium. The latter flares outward and becomes indistinct as it approaches the chorionic membrane, so that in this region the parietal mesoblast is more irregularly marked off from the exocoelom, constituting an area of scattered mesoblastic tissue in which the exocoelomic development is not yet complete.

A section through this region is shown in figure 37, plate 6, in which will be recognized the chorionic membrane above and a portion of the yolk-sac of the main embryo and its abdominal stalk below. In the loose tissue lying between these are the two detached vesicles, which together constitute the twin. The smaller, with a lumen 0.03 mm. in its largest diameter, is interpreted as the yolk-sac. It is shown under greater enlargement in figure 36; here it can be seen that its wall is made up

<sup>1</sup> In the Linzenmeier specimen, owing to the excessive and irregular character of the menstruation, the data could not be safely used.

of a thick sheet of protoplasm interspersed with a single layer of nuclei. This endodermic layer is inclosed by an irregular membranous layer of mesoderm.

A series of sections through the larger structure, which is interpreted as the amniotic vesicle, is shown in figures 29 to 35. This vesicle, the inside diameters of which are 0.1 by 0.1 by 0.06 mm., consists of an ectodermic layer clearly subdivided into embryonic ectoderm and amniotic ectoderm. The embryonic ectoderm forms a sharply marked-off plate on the side towards the body-stalk of the larger embryo. Like the yolk-sac, it is surrounded by a membranous sheet of mesoderm in which here and there are vesicular arrangements of its cells, in some instances closely resembling empty young blood-vessels.

In accepting this structure as a monozygotie twin, the discrepancy in size between it and the principal embryo suggests the possibility of its being stunted. Whether it is essentially normal in form and simply retarded in its development can only be determined by comparison with a much larger group of specimens than is to be found in the literature up to the present time. The amniotic vesicle seems to be well preserved and shows relatively normal differentiation. It corresponds in many respects to those seen in the ova described by Peters, Fetzner, Jung, and Strahl and Beneke. Our specimen differs from these, however, in the complete detachment of the yolk-sac. This condition might justify us in considering it as abnormal. It will be recalled, however, that the amniotic vesicle and the yolk-sac were as widely separated in the Bryce-Teacher ovum which has hitherto been regarded as normal. Inasmuch as the twin in the Mateer specimen is at least stunted and possibly abnormal, it would cast some doubt upon the probability of a detached yolk-sac ever being a normal condition.

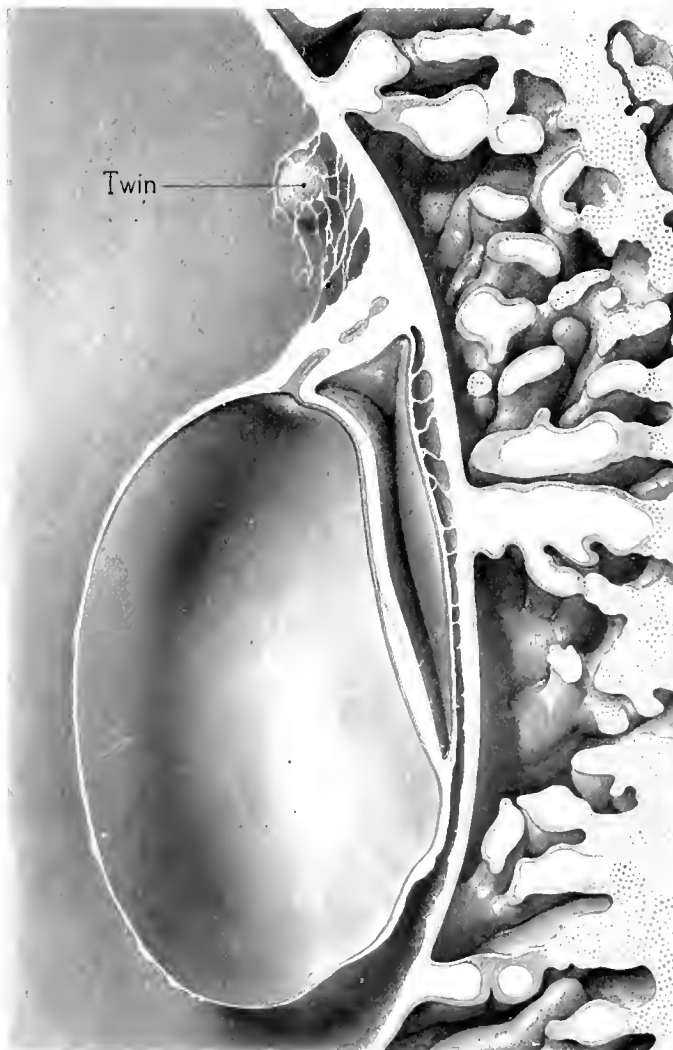
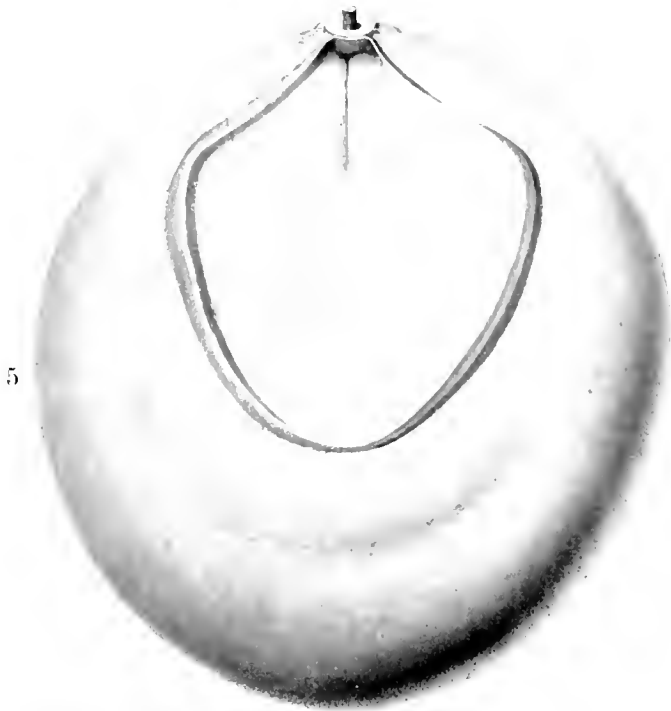
If the pregnancy had not been terminated in this case it is probable that the larger embryo would have gone on to maturity, and that the smaller one would have remained stationary in the form of two small epithelial vesicles, and so would have been entirely overlooked. Careful search at the placental attachment of the umbilical cord might frequently reveal the presence of similar minute epithelial vesicles, the remains of stunted twins, and thus we might find that the tendency toward twinning in man is even greater than is now supposed.

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PLATE I.

- FIG. 5. Frontal view of wax-plate reconstruction of Mateer embryo, showing yolk-sac and embryonic plate outlined by the cut edge of the amnion, which is shown as removed. The stump of the body-stalk can be seen with the allantois at its center. Extending forward from the caudal end of the embryonic plate is a well-defined primitive groove. Enlarged 60 diameters.
- FIG. 6. Median view of wax-plate reconstruction of Mateer embryo showing the form of the amniotic cavity and its relation to the chorionic membrane and the yolk-sac. The allantois projects through the body-stalk and is interrupted near its center. In the loose tissue caudal to the body-stalk is an epithelial mass constituting the amniotic vesicle of the twin embryo. Enlarged 60 diameters.
- FIG. 7. Photograph of section through embryo No. 763, Carnegie Collection. This specimen would belong among those in our group 1 having branched villi. The embryo, consisting of two vesicles, can be seen in the center. The series being incomplete, the definite form of the embryo can not be made out. This specimen is distinctly younger than the Mateer specimen and its villi are to be compared with those in figure 8. Enlarged 30 diameters.
- FIG. 8. Photograph of a typical larger villus of the Mateer specimen, showing transition into trophoblast. For a detail of these structures see text-figure 4. Enlarged 50 diameters.

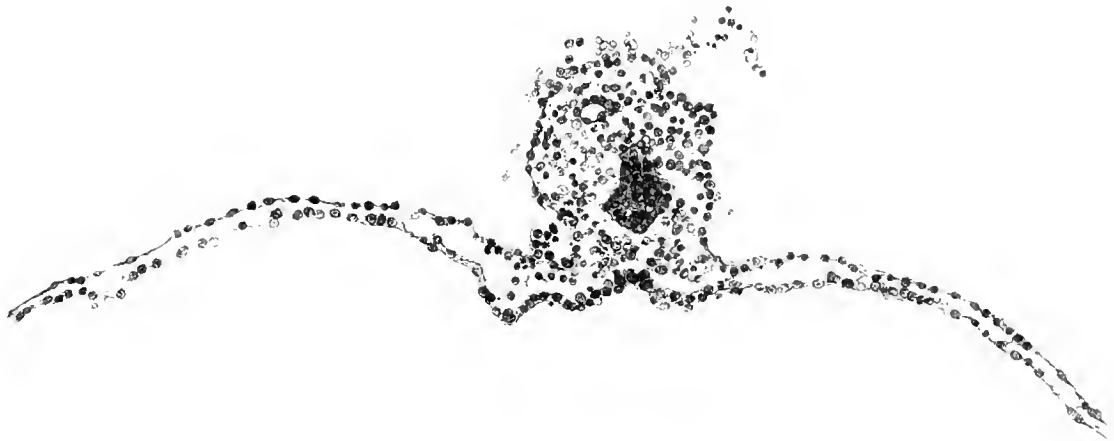




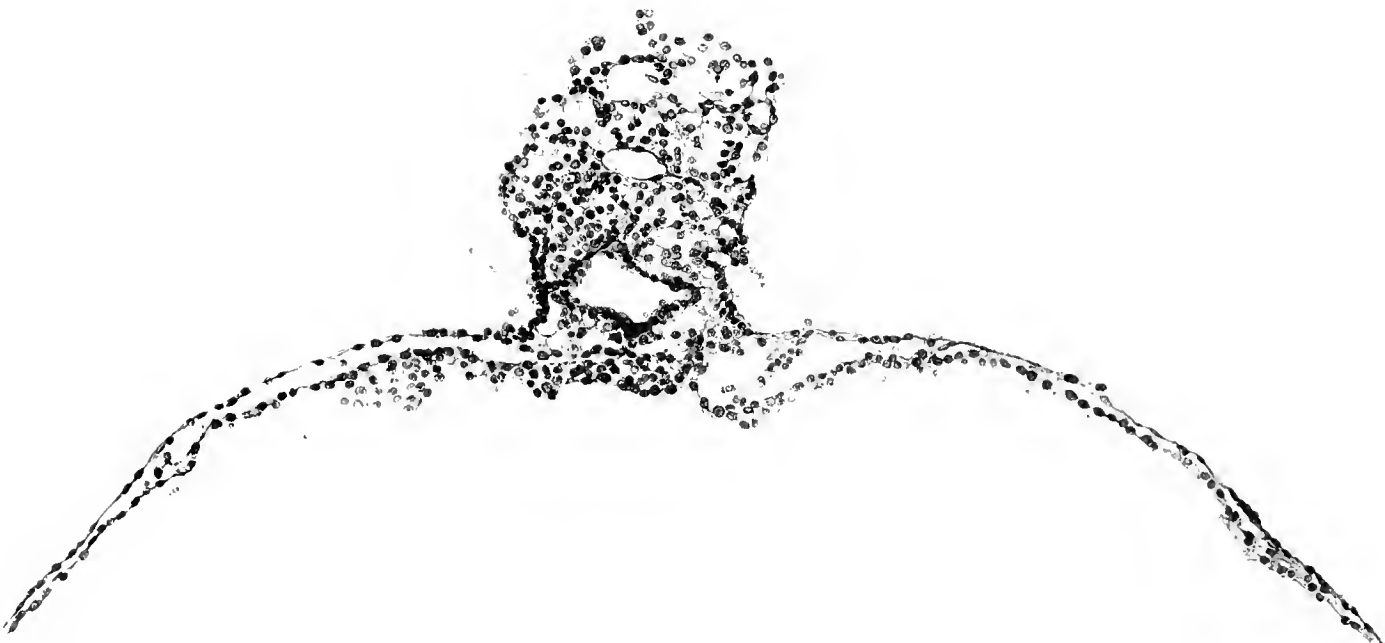




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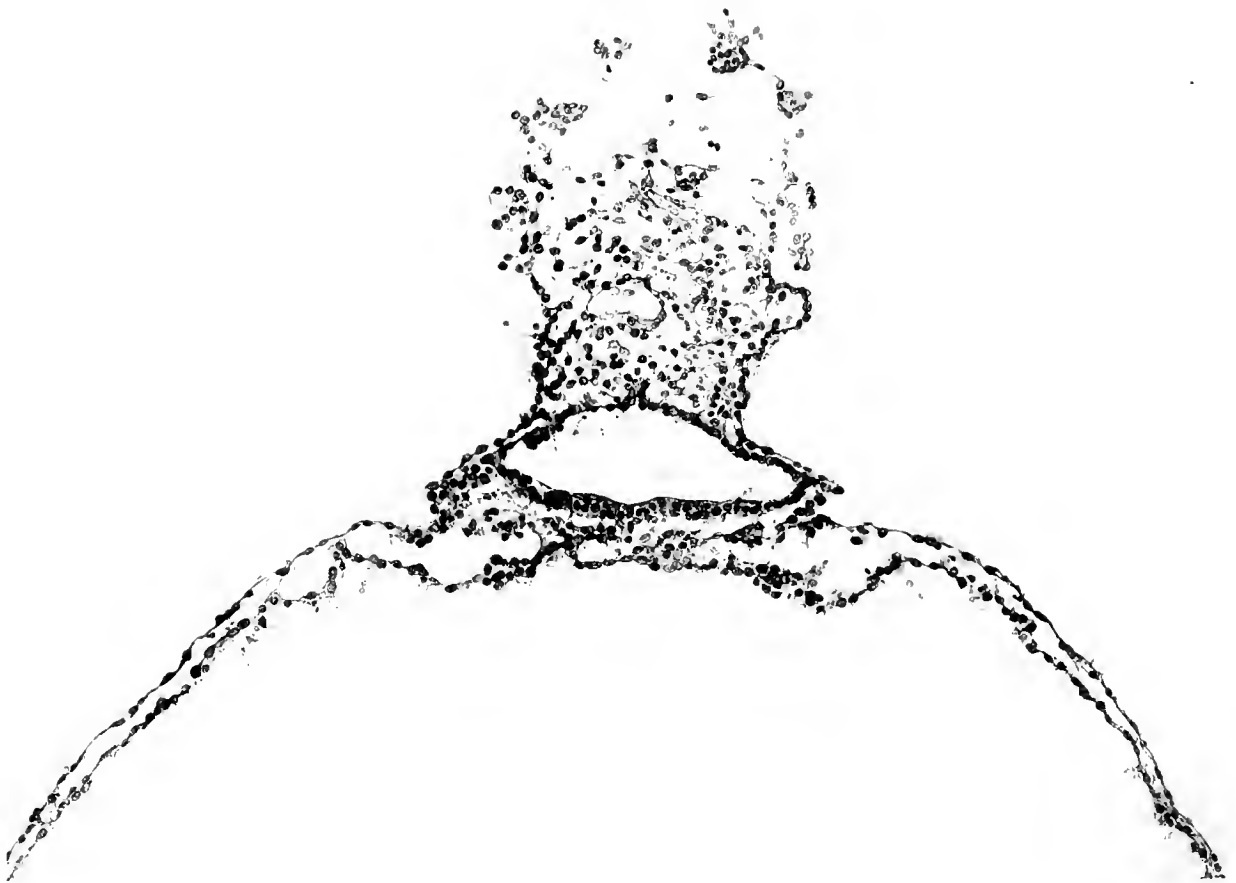
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FIGS. 9 to 16, shown on plates 2, 3, 4 and 5, represent detailed drawings of typical sections through the region of the embryonic plate indicating the arrangement of the three germ-layers. The section numbers are as follows: Fig. 9, Section No. 78; Fig. 10, Section No. 75; Fig. 11, Section No. 70; Fig. 12, Section No. 67; Fig. 13, Section No. 64; Fig. 14, Section No. 59; Fig. 15, Section No. 56; Fig. 16, Section No. 50. All of the figures are enlarged about 180 diameters.



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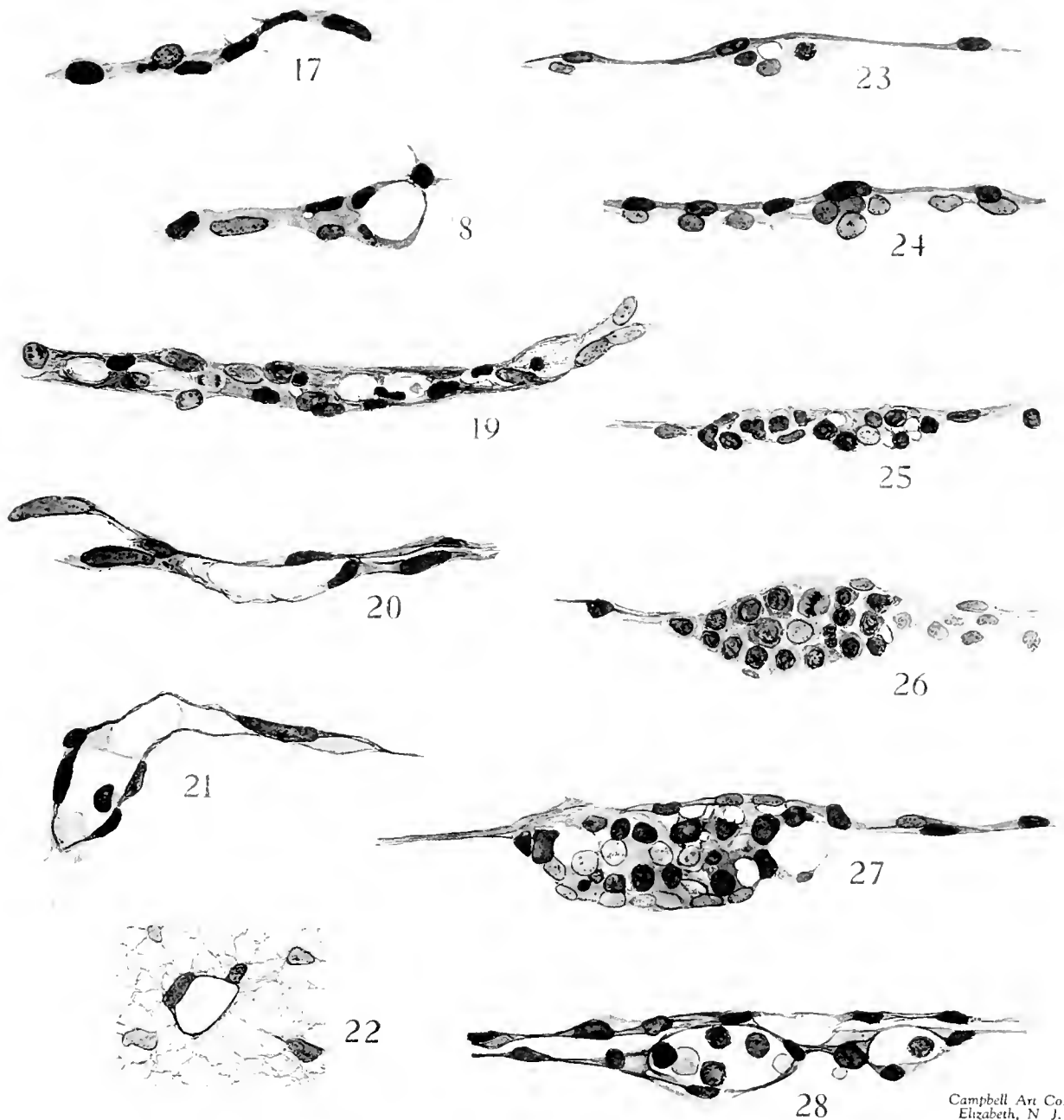
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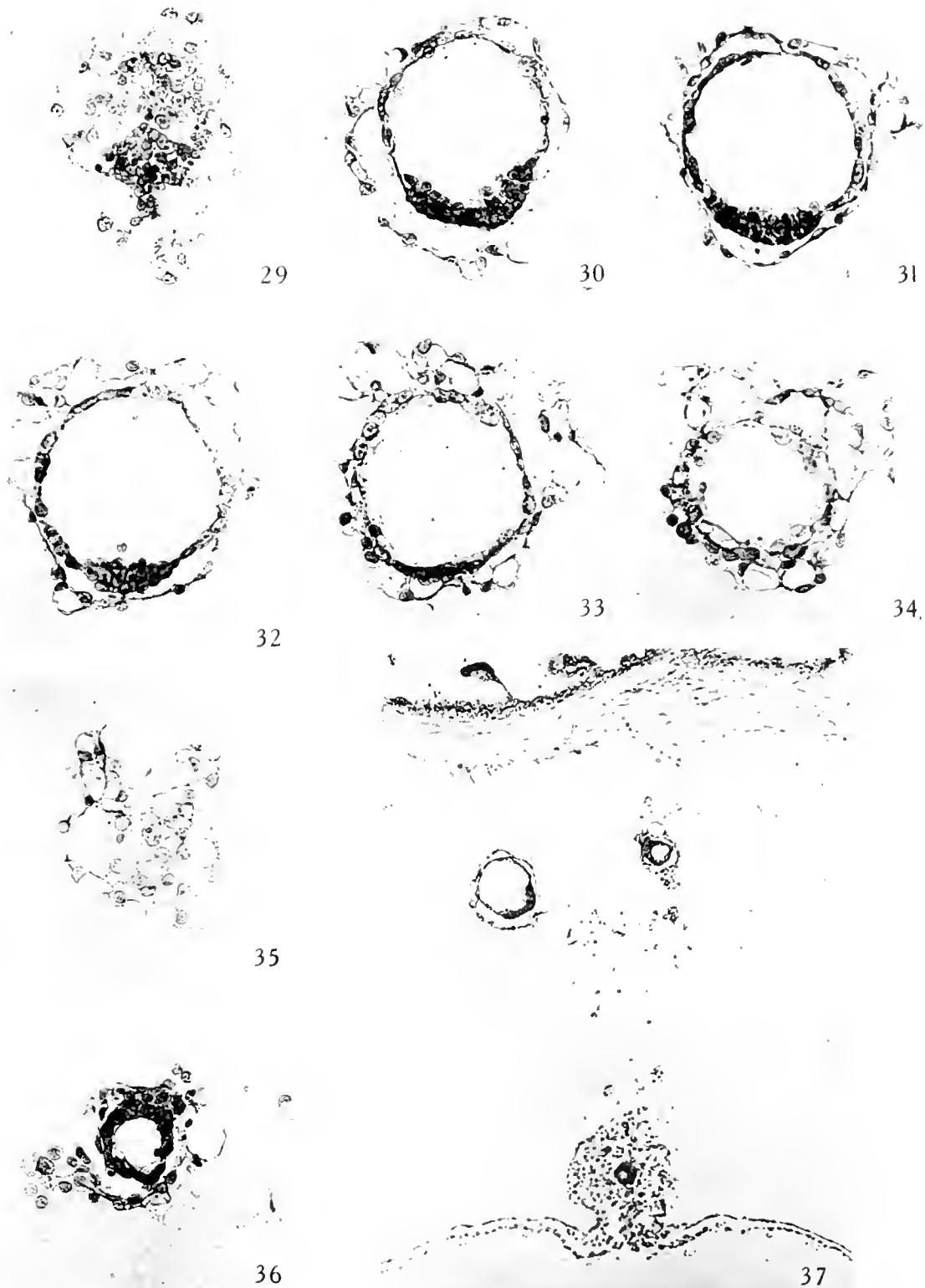


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Elizabeth, N. J.

FIGS. 17 to 22. Sketches of selected cell groups found in the chorionic villi, which are interpreted as blood-vessels in a progressive series of developmental stages. In figure 22 is shown the relation of the endothelium to the surrounding stroma. Enlarged 600 diameters (approx.).

FIGS. 23 to 28. A similar series of angioblastic cell-groups found in the wall of the yolk-sac. In this series the endoderm is shown as the upper and the mesoderm as the lower layer. Enlarged 600 diameters (approx.).





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FIGS. 29 to 35. Photographs of serial sections through the amniotic vesicle of the twin embryo, showing the ectodermic character of its wall and the investing layer of mesoderm. In figures 30 to 33 the region of the amniotic plate is clearly marked off. Enlarged 300 diameters.

FIG. 36. Photograph of section through the yolk-vesicle. Enlarged 300 diameters.

FIG. 37. Section (No. 73) through region of abdominal stalk, showing the relation of the two vesicles (constituting the twin), to the larger embryo and the chorionic membrane. Enlarged 112 diameters.



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CONTRIBUTIONS TO EMBRYOLOGY, No. 44.

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THE EXPERIMENTAL PRODUCTION OF AN INTERNAL  
HYDROCEPHALUS.

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With two plates.

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# THE EXPERIMENTAL PRODUCTION OF AN INTERNAL HYDROCEPHALUS.

BY LEWIS H. WEED.

## INTRODUCTION.

The pathological condition of internal hydrocephalus is intimately connected with abnormalities of the production or absorption of cerebro-spinal fluid. The first explanation of this condition—an increased elaboration of the fluid by the choroid plexuses—remains a hypothetical possibility. Obstruction to the normal pathways of drainage of the fluid has been many times demonstrated anatomically, so that now this idea of the origin of the disease may be considered to rest on a firm basis. It is with the experimental production of that type of hydrocephalus, due to obstruction to the flow of the cerebro-spinal fluid through its normal channels, that this paper will deal.

To insure a more complete understanding of the anatomical problems underlying internal hydrocephalus, a short review of the essential morphological and physiological features of the pathways of the cerebro-spinal fluid will be given. The conception that the greater part of the cerebro-spinal fluid is produced by the choroid plexuses of the cerebral ventricles is no longer strongly controverted; when first advanced, it had only the glandular histology of the plexus to support it (Faivre, 1853; Luschka, 1855), but much later a combination of pharmacological, histological, and pathological observations indicated beyond question the essential rôle of these plexuses in the elaboration of this body-fluid. From a summary of this evidence and from personal study Mott (1910) suggested the term "choroid gland" for these vascular structures. More recently embryological studies related the first extraventricular flow of the fluid to the initial tufting of the plexuses (Weed, 1916 *a* and *b*; 1917). Pathologically, the production of an internal hydrocephalus by tumors occluding the ventricular passages has been recognized for years as affording evidence of an intraventricular source of the fluid.

From the choroid plexuses the fluid is poured into the cerebral ventricles which are lined by ependymal cells of ectodermal origin. The fluid from the lateral ventricles escapes through the foramina of Munro into the third ventricle, thence through the aqueduct of Sylvius into the fourth. Additions to the fluid are made by the choroid plexuses of the third and fourth ventricles. From the fourth ventricle this fluid passes into the mesodermal subarachnoid spaces through the foramina of Magendie and of Luschka, if these be actual openings, or at least through permeable membranes if the patency of the foramina be questioned.

The cerebro-spinal fluid leaving the ventricular system, is distributed from the cisterna cerebello-medullaris, which it first reaches on leaving the fourth ventricle. In the comparatively large channels about the base of the brain the flow is rapid and great; down the cord the dispersion is also fairly efficient, but over the con-

vexities of the cerebral hemispheres a very slow and less efficient distribution occurs. Everywhere this extraventricular course is through the meshes of the subarachnoid spaces—a complicated, interrupted fluid-channel, completely clothed by low or flat mesothelial cells. In the cisternal regions, and to a lesser extent about the upper spinal cord, the mesh of this fluid channel is rather large, but over the hemispheres it becomes quite small. The pial reflection of the mesothelial cells lining the subarachnoid space is broken by the openings of the perivascular spaces (Robin); into these cuffs the mesodermal cells continue for a short distance. By way of these perivascular spaces a small but rather important fluid, representing probably the elimination of the brain tissue, is added to the subarachnoid cerebro-spinal fluid.

It seems quite well established that the cerebro-spinal fluid is returned directly into the blood stream. This escape of fluid is wholly from the subarachnoid space; all observers are agreed that the intraventricular absorption is minimal. The most important hypotheses regarding the mode of absorption of the fluid are those which assume the Pacchionian villi as the pathways into the dural sinuses (Key and Retzius, 1876); that the fluid reaches the cerebral capillaries by way of the perivascular sheaths (Mott, 1910); or that a direct absorption into the capillaries of the subarachnoid space occurs (Dandy and Blackfan, 1913, 1914). More recently evidence that the arachnoid villi (normal projections of arachnoidea into the dural sinuses) are the essential structures in the pathway of escape, has been presented (Weed, 1914, *a, b, c.*). The direction of flow (toward subarachnoid space) in the perivascular spaces argues against Mott's idea of drainage, while the absence of capillaries in the arachnoid renders the hypothesis of Dandy and Blackfan untenable. The strongest evidence, both anatomical and physiological, favors the idea of a major absorption from the cranial portion of the subarachnoid space.

With this conception of the pathway of the cerebro-spinal fluid, it becomes evident that the obstruction to outflow of fluid may be either intraventricular or extraventricular. Pathologically, the differentiation of hydrocephalus into two classes with reference to the point of obstruction has repeatedly been made. In the one, the block to outflow occurs usually at the points of constriction of the ventricular system, as at the foramina of Munro, within the aqueduct of Sylvius, or (in rarer cases) within the fourth ventricle. In the second variety of cases the obstruction to flow of the fluid occurs within the subarachnoid space. The mechanism of this intrameningeal block is not well understood, except in the cases of obvious filling of the cisterna cerebello-medullaris with fibrinous exudate, occasioning a macroscopic closure of the exit.

The occurrence of a typical internal hydrocephalus due to block within the narrow portions of the ventricular systems seems of easy explanation. With continued elaboration of cerebro-spinal fluid by the choroid plexuses, and practically no intraventricular absorption, it seems inevitable that dilatation of the ventricles should take place. Such enlargement reaches its fullest expression in the lateral ventricles with compression and thinning of the cerebral cortex. With the increased pressure of the fluid there is ultimately brought about a balance between the production of fluid by the choroid plexuses and the minimal intraventricular absorption, plus other potential agencies of escape.



But when consideration is given to the enlargement of the lateral ventricles from obstruction to flow within the meninges, a plausible explanation is more difficult. The mere mechanical explanation does not fairly meet the question as to why the meningeal block does not cause compression of the cerebral cortex, dilatation of the subarachnoid spaces, and a typical external hydrocephalus. In one variety of this type (obstruction at the foramina of the fourth ventricle) the mechanical explanation holds. But actually, a diffuse block to the outflow of cerebrospinal fluid within the meninges results almost inevitably in the later production of an internal hydrocephalus.

Clinically, a differentiation between the two types of internal hydrocephalus may be made. In children Cushing (1908) found that the cerebral ventricles of one kind of internal hydrocephalus (due to block in the meninges) could be drained by lumbar puncture, while in the other (due to block in the ventricular system) the cerebral ventricles could be emptied only from the ventricular needle. Similarly, Dandy and Blackfan (1913, 1914) recovered by lumbar needle phenolsulphonophthalein injected into the cerebral ventricle in the "communicating" type, while the subarachnoid absorption was markedly retarded. In the obstructive type these writers showed a negligible absorption from the cerebral ventricles, but an unimpaired absorption from the subarachnoid space. In these cases the dye injected into the dilated ventricles did not appear as normally in the lumbar fluid.

Experimentally, the obstructive type of hydrocephalus alone seems to have been produced. Dandy and Blackfan (1913, 1914) were able to cause a typical internal hydrocephalus in dogs by two methods, the first of which gave the pathological picture of an intraventricular block. Pledgets of cotton were introduced from the occipital region through the fourth ventricle into the aqueduct of Sylvius. Such a foreign obstruction caused signs of cerebral pressure (lethargy, vomiting) and a rather acute dilatation of the ventricles was produced. In a somewhat different way the same enlargement of the ventricles was accomplished by ligation of the vein of Galen, but this occurred in only one of the ten animals used. In the other nine animals the higher ligation apparently permitted sufficient collateral circulation. Dandy and Blackfan used young dogs 2 to 6 months of age; at this time in the dog the cranial sutures are strongly united, so that no enlargement of the head occurred. The time allowed for the dilatation of the ventricles varied from three to eight weeks after the operation. At the end of this period a fair degree of hydrocephalus was present.

At about the same time Thomas (1914) published the results of his experiments on the production of an internal hydrocephalus by the intraventricular injection of aleuronat in starch. This protein caused a marked inflammatory reaction, blocking finally the ventricular pathway at one or other of the narrow parts. Dogs were used throughout for the observations. Thomas found little enlargement of the ventricles present in the first week, but in the chronic stage of the inflammatory process incited by the protein, an internal hydrocephalus developed, with symptoms of increased intracranial pressure. The dilatation of the lateral ventricles occurred slowly and reached its maximum in about two months. Subsequent intra-

ventricular injection of India ink demonstrated that the obstruction to flow of the cerebro-spinal fluid occurred, in the different animals, either at the foramen of Munro, in the aqueduct of Sylvius, or most frequently, at the foramen of Magendie. It must be emphasized that in many cases the ventricular dilatation was markedly asymmetrical (the greater enlargement being on the side of the injection) and that the ventricular wall was often irregularly eroded. The intraventricular accumulation of fluid was associated with a sterile inflammatory process.

An attempt to produce an internal hydrocephalus in kittens was made by Burr and McCarthy (1900). These authors described a case of internal hydrocephalus in man with a macroscopically boggy ependyma. Microscopically, the sections showed proliferation of ependyma and neuroglia, interstitial infiltration of the choroid plexuses, and some perivascular invasion with inflammatory cells. As the nature of the lesion in this case suggested to these workers a toxic irritant, intraventricular injections of sterilized urine, glycerine extract of the adrenals, tuberculin, hydrochloric and carbolic acid were made in kittens. No dilatation of the cerebral ventricles occurred in these animals, though similar ependymal and glial changes were reproduced.

Flexner (1907) recorded the occasional production of an internal hydrocephalus in monkeys following lumbar subarachnoid injection of meningococci. Of one subacute case of meningitis he wrote:

"A striking feature of the sections is derived from the width of the ventricles. As a rule, these appear as slits in the sections; in this case they are wide cavities. Usually, the ependymal epithelium is regular and relatively high; in this case, it is often depressed or flattened, and a considerable flattening of the choroid plexus, toward the wall of the ventricle, is noticeable. A considerable degree of sub-epithelial cellular proliferation has taken place in the walls of the lateral and fourth ventricles. Leucocytes are moderately abundant in the ventricles."

#### METHOD OF INVESTIGATION.

The method of investigation in this study developed out of the idea that it would be possible to produce an internal hydrocephalus in animals if a sterile meningitis of appropriate type and distribution could be caused. This assumed that the meningeal variety of obstruction to flow of the cerebro-spinal fluid was an experimental possibility and the agencies were selected with this end in view.

In a previous report (Weed, 1917) notation was made of the fact that, following the subarachnoid injection of inert carbon particles, evidence of phagocytosis on the part of the cells lining the subarachnoid space could be made out. By increasing the quantity of this inert substance it was hoped that a more extensive reaction of inflammatory cells and of the arachnoidea could be brought about. Quite similarly it was proposed to make subarachnoid or intraventricular injections of other insoluble particles. It was desired primarily to reproduce a hydrocephalus in a very young animal, so that a typical enlargement of the head, comparable to the fairly common condition in children, might be produced. To accomplish this, it was felt that the experimental procedure must be necessarily simple and uninvolved.

Fortunately almost the first technical procedure resulted in the production of a typical internal hydrocephalus. A litter of kittens two weeks old was obtained;

a 5 per cent suspension of lampblack in Ringer's solution was injected into the lateral ventricle of one animal, and the others of the litter were used for injection of other materials or for control. The head of the kitten receiving the intraventricular lampblack enlarged; the fontanelles widened markedly and a typical clinical picture of the desired condition resulted.

Subsequently the technical procedure was modified in many ways. Experiments demonstrated that a somewhat more pronounced pathological change, with less disturbance of the kittens' activities, could be produced by subarachnoid injection of the lampblack through a needle in the occipito-atlantoid ligament. This latter method alone was employed in the experiments on adult cats. In the earlier cases two intraventricular injections of lampblack were sometimes given with an interval of several days between; later it was found that this double injection was useless, provided the concentration of the carbon in the initial injection were great enough. Customarily, in both cats and kittens, as much cerebro-spinal fluid as possible was allowed to escape from the occipito-atlantoid or ventricular needle; injections of 1 c.c. (in kittens) to 10 c.c. (in adults) of a 5 to 10 per cent suspension of lampblack in Ringer's solution were made through the needle after the release of fluid. The lampblack originally injected was found to be more efficacious than any other sample tested; it is sold under the trade name of "Germantown Black" and is manufactured by the L. Martin Co., New York City. Other lampblacks produce a similar condition of hydrocephalus, but not as rapidly or as invariably as does this Germantown Black. Whether this difference in reaction is due to difference in size of granulation or to some other "binder" in the carbon is not known.

The intraventricular or subarachnoid introduction of these carbon granules was controlled by similar injections of equal amounts of other insoluble granules in suspension. Because of its wide employment as an injection-medium, cinnabar (red mercuric sulphide) was selected as the routine control granule. None of the particular insoluble substance used for this purpose gave rise to an internal hydrocephalus, but it does not seem improbable that other insoluble granules may be found to produce such a pathological change.

A point of considerable practical importance in the care of kittens subjected to experimental procedures concerns the return of the animals to the mother without apparent change. In these experiments the hair was not shaved in the area of puncture and the kittens were returned to the mother only when fully recovered from the anesthesia. With these simple precautions it was possible to inject kittens one day of age and to have the mother subsequently take care of them as well as the others in the litter. In general, kittens up to four weeks of age were subjected to the experimental injections; these gave the greatest enlargement of the cranium and remained fairly well for the greatest lengths of time.

At the end of varying periods the animals (both kittens and adult cats) were sacrificed and injected with 10 per cent formalin through the aorta, as were those dying from the experimental procedure or from bronchial infection (a common difficulty in such animals). After hardening for a suitable period further macroscopic studies were made and the heads were sectioned.

## THE REACTIONS OF THE EXPERIMENTAL ANIMALS.

Age is apparently a determining factor in the reactions of an animal after the intraventricular or subarachnoid injection of lampblack. Probably more important than the actual age of the animal is the associated degree of ossification of the skull; if the union between the bony plates is marked and firm, the increase in intracranial tension does not result in any enlargement of the bony skull. This enlargement of the head, met with in kittens after the subarachnoid or intraventricular injection of suspensions of lampblack, seems to be compensatory in nature and allows much more cerebral function than is possible in an adult animal with a rigid skull. The experimental findings in the immature and in the adult animals will in consequence be detailed under separate headings.

## KITTENS.

In all, 35 kittens were given subarachnoid or intraventricular injections of suspensions of lampblack. These kittens were from 18 litters and at the time of experimentation were normal, healthy animals. The age at the time of experimentation ranged from 24 hours to 42 days. The younger animals were all being taken care of by the mothers, whereas the older in the series were being fed as adults.

As the technical procedure of injection was very simple, practically none of the animals died acutely as the result of the experiment. Of the 35 kittens used, 8 died (or were sacrificed because of poor physical condition) within the first 8 days; 8 survived for more than 4 and less than 10 days; and the remainder (19) lived for over 10 days. The longest period of survival after the experimental injection was 47 days. The cases considered to be most successful and showing most pronounced enlargement of the head were those which lived more than 14 days after the experimental injection. The number of the animals in the series surviving for several days is really surprisingly large, and the proportion of early deaths, considering the age of the animal at the time of the experiment, was very low.

No real or essential difference between the reaction of the kittens receiving subarachnoid or intraventricular injections could be made out. Possibly, those receiving the lampblack into the subarachnoid space showed enlargement of the head somewhat more rapidly and remained in better physical condition for a longer period. By both routes, however, the production of an internal hydrocephalus seems equally certain, provided a suitable dose (best, about 1.0 c.c. of a 10 per cent suspension) of lampblack be given. The release of cerebro-spinal fluid by needle through the occipito-atlantoid ligament is much more certain than is ventricular puncture through the fibrous fontanelle or through the thin bone of a young kitten. Pathologically, there is practically no difference in the hydrocephalus resulting from either procedure; the amount of carbon particles found in the cerebral ventricles is, however, much smaller in the animals receiving the occipito-atlantoid injection. In these experiments the most extreme cases of internal hydrocephalus have been those produced by such subarachnoid injection. Of 35 animals, 19 were given intraventricular and 16 subarachnoid injections.

On recovery from the ether, these experimental kittens with subarachnoid or intraventricular injection can hardly be told from the normal control animal in the litter. If abnormal at all, they tend to be somewhat less active in crawling about and seem cautious in movements, particularly in regard to the head. The next morning the experimental animal usually did not differ from the control, though a slight carefulness and slowness in reaction might be present (noted in 5 out of 16 cases). On the second morning, in the more pronounced cases of obstruction, an enlargement of the head has been made out; this is usually demonstrated by the formation, again, of suture lines previously closed. In other cases no signs of an incipient hydrocephalus were definite until the fourth morning, and this may be considered to be the usual interval before the pathological change may be made out. Thus, aside from a slight initial slowness in reaction, it may be assumed that no abnormalities are apparent until the lapse of sufficient time for the increase in intraventricular tension to cause enlargement of the head.

As soon as definite changes in the size of the anterior fontanelle and in the widening of the sutures have appeared, the further enlargement of the head progresses with a fair degree of rapidity. In those kittens in which injection has not been made until after bony union of the cranium has occurred, there is a definite diastasis of the bones with the forming anew of fibrous sutures and fontanelles. This opening-up of the bony skull has been observed in 5 kittens, the ages at the time of injection varying from 17 to 42 days. In general, however, these experiments have been performed on animals in which the closure of the skull was incomplete; the enlargement of the head could be brought about by dilatation of the existing fibrous sutures.

This enlargement of the head is of amazing rapidity and degree. In these experimental cases, as in man, the increase in size is practically entirely confined to the cranial vault, while the base of the skull remains fairly constant. Such enlargement of the vault naturally causes separation merely of the flat bones of the calvarium and leaves unchanged the relative positions of eyes and ears.

The subsequent clinical course of the experimental kittens was largely influenced by the factors mentioned. The weaker kittens soon reached a stage when the head became too heavy for them to lift, so that progression was accomplished by pushing the head along the floor of the cage. In others, the increasing weight of the head could be handled more easily, though most of these showed an ataxia more profound in degree than usual for kittens of equal age. Many of these kittens with enlarged heads remained very active even after two weeks; one in particular continued to climb with great facility upon the perpendicular wire side of the cage. In general, however, it must be granted that the kitten receiving the lampblack was more cautious and more sluggish in reaction than the control. The animals of the same litters subjected to subarachnoid or intraventricular injections of cinnabar could not be distinguished from normal.

The cause of death in the animals was variable, though two agencies were responsible for the majority. In the first place, death of the kittens from infection with *B. bronchisepticus* was unfortunately very common; the bronchial pneumonia

from this organism affected many of the best litters. Again, the kittens with the unwieldy heads were unusually apt to fall and receive injury to the head, etc. Others finally reached a point apparently when the increasing cerebro-spinal pressure affected the medullary centers.

The protocol of a typical experiment with injection of a suspension of lamp-black into the subarachnoid space of a kitten is given below:

Litter of 4 kittens ( $N_3$ ) born May 22, 1918.

When 20 days old (June 12, 1918) the whole litter was in excellent physical condition. Three of the litter were used for experimental injections and the fourth used as control. The history of one of the experimental animals (No. 41) and the control will be given.

The experimental kitten was on this day given ether and without shaving hair, puncture through the occipito-atlantoid ligament was done with release of about 0.5 c.c. clear cerebro-spinal fluid. Through this needle a subarachnoid injection of 1.0 c.c. Ringer's solution containing a 10 per cent suspension of lampblack was made. At the time of injection the fontanelles of all of the litter were closed and the whole skull bony, as determined by palpation. The litter was returned to mother.

On the next day (June 13) the experimental kitten was in excellent condition and its skull still tightly closed. The control was normal and active.

The second morning, however, a difference could be made out between the kittens. The experimental animal's cranial bones were definitely separated, but with a narrow fibrous interval. The control remained as before.

On the third day it was noted that the experimental kitten could not lift its head from the ground, though apparently in excellent physical condition. The animal revolved about its enlarged and heavy head as about a fixed point. The anterior fontanelle was extremely widely opened and the longitudinal and transverse sutures about 3 mm. wide.

This enlargement of the head continued, with the suture lines widening to 4 mm. and the fontanelle becoming still larger. The convexity of the calvarium between the ears increased and the forehead became high and prominent. On the fifth day it was noted that the lower lid was being pulled up, covering the lower half of the pupil. The control animal remained normal and active, its skull remaining bony and enlarging as that of any normal animal.

On the eighth day the following notation regarding the experimental kitten was made: "Same excellent general condition. Head has become very large and forehead is extremely prominent and high. Eyes are fast becoming obscured by the pulling-up of the lower lid; the sclera constantly shows as a white crescent above. Fontanelles and sutures are becoming larger each day, bulging and protruding somewhat. The kitten can just lift its head up but a profound ataxia characterizes all movements. Can progress only by pushing its head along the ground." On the same day the control was recorded as "Excellent shape. Active. Fontanelles tightly closed; bony skull."

The animal continued to gain in strength and was able to raise its head and move around fairly readily though with considerable ataxia. On the twelfth day it was noted that "from the glabella the forehead rises almost perpendicularly. The sutures are palpable from glabella posteriorly to occiput; laterally they may be traced far down under temples. All these suture-lines are from 6 to 8 mm. wide while the fontanelle has a diameter of 10 mm." A photograph (fig. 11) of the animal on this day (June 24, 1918) is reproduced. Again, the next day, "Bony edges of the former calvarium are very ill defined and small. Whole head seems soft and fibrous. Orbital ridge almost obliterated." The control remained normal.

Unfortunately the animal developed a very acute bronchisepticus infection and was sacrificed, with the normal control, on the fourteenth day (June 26, 1918).

As the enlargement of the head is the most striking abnormality to be made out in the kitten during life, a description of this anatomical change will be included. The first noticeable development, as already mentioned, is the alteration in the suture lines. The opening or enlargement of a suture is usually to be demonstrated by gentle palpation; a definite increase in the cranial vault has been noted with certainty on gross inspection only after the fourth day, though before this time palpation gave good assurance of this increase. The enlargement of the head was usually made obvious by alteration in the angulations; this was due to the lack of associated expansion in the base of the skull. One of the first readjustments to this increase in size of the cranial cavity was the elevation of the line of the forehead so that the profile rose abruptly from the line of the nose. This is shown in several figures (No. 11, from a kitten during life, and also Nos. 6 and 9). In the normal kitten the line of the skull in profile slopes backward in a very gentle angle of ascent, while the kitten with the intraspinous lampblack shows an increasingly abrupt rise to the forehead (fig. 9). In the course of about 2 weeks the enlargement of the vault is so great that on front view the forehead seems to tower above the orbital ridge (figs. 5 and 6).

This increase in size of the vault with marked elevation of the forehead is associated with other equally characteristic features. The elevation of the vault proceeds in these kittens so rapidly that the orbital ridge gradually seems displaced backward or obliterated (figs. 5 and 11). As the growth of the base of the skull proceeds as normally in these kittens, the increased intracranial tension continues to pull upward the restraining portions of the base. Laterally, however, over the ears, the whole vault bulges markedly also and overhangs more prominently the bony canal (figs. 7 and 9). Here the bulging is more or less opposed to the retraction of the bony orbital ridge. The result of these forces is the rounding up of the whole cranial vault and to a lesser extent of the base of the skull, with an increase in the transverse diameter and a relatively smaller increase in the sagittal. This apparent rounding-up is shown in figures 10, 13, and 17.

These alterations in the shape and size of the cranium have other readjustments of the general appearance of the animal associated with them. Most striking of all is the general appearance of bulging of the whole head due to an increased convexity between ears and between occiput and glabella (cf. figs. 7 and 11). In the extreme cases the whole head may resemble the typical "Turmschädel" noticed clinically in man. The ears seem in consequence to be placed at relatively low level in the skull on account of the fact that the enlargement has been wholly above the base (figs. 6 and 11).

Quite similarly caused is the pulling upward of the skin from the face and lower part of the head by the enlargement of the vault. This results in the typical white line of sclera showing above the iris and in the obscuring of the lower half of the pupil by the lower lid. With the obliteration of the orbital ridge, the sclera beneath the retracted upper lid appears as a wide crescent, best to be seen from above because of the retracted orbital ridge. This can be made out in figures 7 and 11.



From such findings and changes in the head, as given in the foregoing paragraphs, the diagnosis of an internal hydrocephalus in these kittens has been very easy during life. The kittens show practically every sign noted in the more chronic cases in children. Palpation of the head gives unmistakable evidence of fluctuation of a fluid; pathologically the diagnosis is confirmed. Examinations of the fundus of these kittens' eyes have been attempted but have not proved satisfactory. The control animals of the same litter (subjected to no experimental procedure) have shown no variation from the normal, nor have those other control animals, subjected to similar injections of other insoluble granules (usually cinnabar, fig. 8). Thus each experimental kitten was controlled by an unoperated animal and by another in which analogous granules were injected. The relation of the injection of lampblack to the later occurrence of hydrocephalus seems established.

#### ADULT CATS.

All of the adult cats used in this series were given subarachnoid injections of lampblack through lumbar or occipito-atlantoid needle. No ventricular injections were made, as interest was chiefly lodged in the production of hydrocephalus from intrameningeal injection. In all, 18 adult cats were subjected to such subarachnoid injections; of these, 12 received lampblack; 4 cinnabar; 1 lycopodium; and 1 "carbon flour." Of the 12 receiving the regular lampblack, 4 died within the first 5 days after the experimental procedure. The longest period of survival after the injection was 22 days. On the other hand, the cat injected with "carbon flour" lived 4 months before being sacrificed, while the cat given lycopodium into the subarachnoid space was not killed until after 5 months. The cinnabar animals varied in length of life after injection from 3 days to 2 months; most of these were sacrificed at the time when the lampblack animal in the same series died. Thus, the shorter period of survival in cats receiving lampblack as compared to others in the series is striking.

The difference in reaction of the cats to such subarachnoid injections, as compared to the kittens, can be made out by observation of the animals soon after injection. For the most part, signs of increased intracranial pressure were present the next morning after the injection; the animals would be lethargic, sleepy, and could not be roused. In the more profound cases the animals were found lying on their sides and finally went into coma. The onset of these signs of intracranial pressure frequently occurred within 12 hours; in all of the severe cases the phenomena were obvious within 24 hours. In such experiments, from the experience with kittens, it must be assumed that there is no possible enlargement of the cranial vault or other compensation for the acute increase in intracerebral pressure. The results, in consequence, are not as interesting or as striking as in the kitten, where compensation is possible.

Some of these adult cats with such an experimental, acute obstruction to the flow of cerebro-spinal fluid went through a stage of marked cerebral excitation. The protocol of one such is given below, as it illustrates graphically the onset of the acute pressure-increase and the later conversion into the stage of lethargy and helplessness.



Protocol of Cat No. 99, adult male.

December 17, 1917, 10:35 a. m. Under ether anesthesia, occipito-atlantoid puncture, with release of about 1 c.c. clear cerebro-spinal fluid, was done. Slow subarachnoid injection through this needle of 5.0 c.c. of a 5 per cent suspension of lampblack in Ringer's solution. Normal and rapid recovery from ether; animal walked about room within a few minutes.

One hour later (11:45 a. m.) animal was returned to cage, active and playful. Ran about as normal animal.

Six hours later (5 p. m.) cat was in same active, excellent condition. Ran about cage very rapidly.

Ten hours later (9 p. m.) the animal was seen in a condition of cortical excitation. The movements were usually rotatory in character, though always associated with convulsive retraction of neck. Epileptiform jactitation of all four legs and of the body. Cat can move about only in intervals of quiet between the attacks.

The next day (Dec. 18) the cat was very lethargic and drowsy when undisturbed, but it could be stimulated to run, when it staggered considerably. It was noted, "quite a typical case of developing acute hydrocephalus."

Animal remained lethargic, slow, and sleepy, but was able to move about quite well if necessary. Quite inactive. On the sixth day the animal began to show a characteristic weakness and droop in the movements of his hind-legs, with a strange and rather ataxic lifting of the feet. Then after a few days (Dec. 29) the cat became "much more lethargic and wobbly, \* \* \* no longer able to walk or struggle along. Lies on side in cage all of the time, weak and ataxic."

The cat did not recover from this condition, but became progressively worse and died on the sixteenth day (Jan. 2, 1918). It was injected with 10 per cent formalin through aorta and the brain removed for study. A photograph of transverse sections of this brain are given in figure 3.

This protocol gives the reaction of the animal to a rather small dose of lampblack. Receiving only 5 c.c. of a 5 per cent suspension, it lived for 16 days, though showing typical signs of an increase in intracranial pressure (lethargy, ataxia, etc.). It has been found that if the concentration of the lampblack in suspension be increased to 10 per cent the hydrocephalus is more acute and striking. Such animals may go almost immediately through the stage of excitement, but show the signs of pressure on recovery from the anesthesia. This phenomenon of an acute increase in cerebral pressure seems the more likely to occur in older animals, though absolute data on this phase can not be had, as the ages of the cats used can be told only approximately. Obviously old cats (as judged by teeth, skin, activity, etc.) have not, as far as this impression holds, shown the same tendency to recovery noted in the young adult animals. To a far greater degree, the age-difference in reaction is brought out in the kittens, as already detailed.

These adult cats, then, after subarachnoid injection of suspensions of lampblack, exhibit during life but little of interest in their reactions. In general, the older animals show, within several hours, signs of disturbance of the intracranial pressure; some pass through a stage of excitement, but most of them become immediately lethargic, weak, and ataxic. Some of these adult animals may recover, after a couple of days, from the acute pressure changes and live for many weeks as fairly normal animals. There is a striking difference in reaction after the injection, and due

to this individuality no general rule can be formulated. The generalizations recorded will remain more or less as impressions, but are of importance in the discussion.

One adult animal subjected to a subarachnoid injection of "carbon flour" (in which the granulation is quite coarse) showed really no clinical signs of acute cerebral pressure, but on being sacrificed after several months exhibited some enlargement of the lateral ventricles. Another cat was given a subarachnoid injection of lycopodium spores (sterilized by boiling). The cat remained normal and active for 6 months; it was then sacrificed for pathological control. No abnormality existed except a widespread distribution of the lycopodium throughout the subarachnoid space (fig. 4). To further control the injection of granules in the subarachnoid space, several animals were given injections of cinnabar, similar in amount to those of lampblack. None of these animals showed any signs of increased intracranial pressure and, post mortem, no ventricular dilatation was present (fig. 2). Another animal was given repeated massive doses of the cinnabar into the subarachnoid space; the result of these repeated huge doses was a gradual abolition of function of the hind-legs, without signs of an increase in intracranial pressure. The peculiar power of the carbon granules in causing increased pressures within the central nervous system, with resulting hydrocephalus, seems therefore indicated.

#### GROSS PATHOLOGY OF THE CONDITION.

The diagnosis of the lesion of the central nervous system after the intraventricular or subarachnoid injection of lampblack, cinnabar and other particulate substances, naturally depends largely upon the pathological picture post mortem, though a good conception of the lesion may be had during life (especially in the kitten). In these experiments all of the animals were injected with 10 per cent formalin through the aorta and the tissues allowed to harden for some time before the initial dissection and final immersion in formalin. The bony skull was then removed in the adults and the brain preserved with the dura intact. In kittens, however, due to the widely dilated sutures, etc., the brain was not removed, but was studied *in situ* in the skull by means of gross sections.

In both kittens and adult cats, in which intraventricular or subarachnoid injections of lampblack were made, the essential pathology of the central nervous system concerns a marked dilatation of the lateral cerebral ventricles with alterations of the cortex, a typical internal hydrocephalus. As the condition is quite different in the younger animals, as compared with the adults, the descriptions of the lesions will be given separately. Control animals, receiving similar injections of other granules, showed no abnormality of the central nervous system.

#### KITTENS.

The gross pathological lesion in the kittens surviving the intradural injection of lampblack for 10 days or over is practically identical in the different specimens. Reduced to simplest form, the abnormality consists in a tremendous and remarkable dilatation of the cerebral lateral ventricles (figs. 10, 14, 16). This increase in size of the lateral ventricles, associated with an enlargement of the kitten's head, results in a marked thinning of the cerebral cortex (figs. 10, 14, 16, and 20). In

some of the cases the third ventricle seems obliterated by its marked enlargement and by the rearrangements of the walls of the interventricular foramina; in others the form of this ventricle is still left, though the whole structure has greatly increased in all of its dimensions (fig. 19). The underlying basal nuclei seem to survive this experimental increase in cerebro-spinal pressure most efficiently; their markings are still wholly visible in the basal view of the sectioned specimen (fig. 16). These general characteristics hold for practically all the specimens obtained. The whole process may be likened to a partial reversion to the embryonic type of cerebral ventricle.

The essential feature of this experimental lesion (the dilatation of the cerebral ventricles) must be taken as the initial, direct result of the increased pressure of the cerebro-spinal fluid; this pressure is, in its turn, to be referred to the obliteration of certain of the pathways of the cerebro-spinal fluid and the consequent damming back of the fluid. For, with the chief production of the fluid intraventricular (by choroid plexuses), and with the obstruction to flow distal to the third ventricle, it seems but natural that the necessary readjustments should occur within the lateral ventricles. The initial increase in the size of these ventricles is the direct result of the increase in pressure, but the enormous dilatation (figs. 10, 14, and 16) met with in kittens is due to the potential distensibility of the head. This permits a tremendous increase in the size of the lateral ventricles, not possible under other conditions; and associated with this relatively extreme dilatation of the ventricles in kittens is the partial rounding-up of the different diverticula of the original cavities. Thus, the body and anterior horn of each lateral ventricle is early consolidated into a general, undifferentiated fluid-container; the posterior prolongation is included at about the same time. The temporal cornu, running anteriorly toward the temporal pole, soon after shows evidence of enlargement in consequence of the increasing pressures, and then takes part in the general process of ventricular dilatation. The more extreme the enlargement, the more the distinction between the temporal prolongation and the main body of the lateral ventricles is eliminated. The net result of these alterations is that, on either coronal or transverse sections, the lateral ventricles, instead of appearing as mere slits as in the control or cinnabar kittens (fig. 10), seem to occupy, with their content of cerebro-spinal fluid, the major portion of the kitten's cranial cavity (figs. 10 and 20).

The thinning of the cerebral cortex is as remarkable and as extreme as the dilatation of the ventricles. Normally, the gray and white matter of the cortex practically fills the cranial chamber, with the exception of the narrow slits of the ventricles. This is shown in gross in the normal control animals in figures 10, 19, and 20. In the kittens receiving either intraventricular or subarachnoid injections of lampblack the cortex becomes, within 10 days, reduced to a thin sheet of nervous tissue 1 to 4 mm. in thickness. For the most part the extreme reduction in thickness is in the parietal and adjacent areas; around the temporal or frontal poles, resting on the more or less fixed portion of the skull, the thinning out of cortex may not be so extreme, though some cases show marked thinning of temporal cortex. This reduction of cortex is striking, and it is remarkable that any cerebral function,

as judged by the imperfect activity of the animal, should persist. In such a thinned cortex it is quite difficult to distinguish between the gray and white matter. It seems, on gross appearance, that the remaining portion of the brain tissue is composed largely of fibers and that the nerve cells are spread out and condensed into a very narrow zone beneath the pia, rendering macroscopic identification difficult in the formalinized preparation.

In practically all of the more pronounced hydrocephalics the basal ganglia may be made out on examination of the inferior surface of the ventricles from above. The same picture of prominent masses of the ganglia is shown on coronal section, but the best views are afforded in the specimens in which the top of the cranium has been removed by a transverse cut. The general appearance of these masses is given in figure 16, from a kitten which was sacrificed 22 days after a subarachnoid injection of 1 c.c. of a 10 per cent suspension of lampblack. On each side two rounded masses project into the enormously dilated ventricles; these represent the more or less undisturbed nucleus caudatus and nucleus lentiformis. The thalamus is obscured somewhat in a gentle swelling in the medial portion of each hemisphere. The dislocation of these nuclear masses is not marked, for they are an integral part of the brain tissue in approximation to the base of the skull; changes here are much less pronounced than in the vertex. Quite similar to this survival of the basal ganglia is the isolation (on development of an internal hydrocephalus) of the fiber bundles of the fornix, for these maintain a strand-like prolongation on the floor of these dilated ventricles. Other fiber-bundles likewise can be made out, dislocated to a greater or lesser extent in the enlargement of the third and lateral ventricles. Such a pushing-back holds for the fibers of the corpus callosum (fig. 20).

There is no essential difference between the internal hydrocephalus produced by intraventricular injection of lampblack and that produced by subarachnoid. Record has been made of minor differences, such as the more or less extensive obliteration of the third ventricle and of the septum pellucidum in the direct intraventricular injections, as compared to the partial survival of these structures in the subarachnoid type. Most marked of all the differentiations, however, is the variation in the distribution of the carbon granules found at autopsy. In the kitten receiving ventricular injection a dense black layer of granules obscuring the picture is customarily found in the basal regions of the dilated cerebral ventricles (fig. 10). In the upper half of the cavity, however, the amount of lampblack is much less. Quite unlike this is the much smaller amount of carbon visible in the dilated ventricles of kittens injected by the subarachnoid route. In these, collections of granules, scattered and small in amount, are the usual findings in the ventricular floor (as in fig. 16), but in some of the specimens the walls of the ventricles are obscured by a deposit giving in the gross a brownish tint.

An interesting feature of the partial obliteration of the basal markings by the dense layer of carbon granules in such intraventricular injections concerns the relations of the choroid plexus. This appears, in the formalin specimen, as a delicate filament in each ventricle, only slightly obscured by the lampblack. These plexuses in some specimens remain fairly free from such carbon deposition, probably due to

the constant secretion of fluid by the cells and the consequent washing-off of any lodging particles. On this hypothesis, which seems justified, the production of cerebro-spinal fluid by the ependyma must be negligible.

The gross pathological features, then, of the central nervous system of a kitten receiving intradural injection of lampblack are those of a typical internal hydrocephalus, such as is clinically quite common in children. The dilatation of the cerebral ventricles thus produced experimentally has been extreme.

#### ADULT CATS.

The same pathological dilatation of the lateral ventricles has been produced by this means in adult animals, but the degree of dilatation is far less than in kittens. This is in large part to be accounted for by the fact that in these adult animals enlargement of the bony skull is impossible and the compression of the cortex, through dilatation of the ventricles, is associated with the physiological limit of increase in intracranial pressure and its consequent bulbar effects. Bitemporal decompressions might possibly allow greater ventricular enlargement and a longer period of survival for the animal.

But by such injections of lampblack an obvious and considerable dilatation of the lateral ventricles is effected (figs. 1 and 3). The lateral ventricles in the most extreme cases have become dilated to 7 to 8 mm. in transverse diameter—an enlargement comparable to those illustrated by Thomas (1914) and by Dandy and Blackfan (1913, 1914), though occurring in a shorter period. The dilatation is for the most part a symmetrical process and is confined at first, or in the less extreme cases, to the body and frontal prolongation of the cavity. In the more striking cases the temporal prolongation is found dilated and the enlargement is traceable into the posterior horn. Such changes in ventricular capacity are wholly similar to the internal hydrocephalus developing clinically in man after obstruction to the pathways of the cerebro-spinal fluid. One receives the impression that the changes in the very old cats are not as marked as those occurring in the young adults.

The thinning of the cortex in these adults is, of course, not marked, and is dependent upon the dilatation of the ventricles; for the reduction in thickness of the cortex is not, under these experimental conditions, in any way primary but is secondary. The differentiation between gray and white matter is not lost, even in these formalinized specimens (fig. 3).

In the adult animals the suspension of lampblack, when introduced into the cisterna cerebello-medullaris by occipito-atlantoid puncture, finds its way into the lateral ventricles in rather small amounts. The chief distribution of the granules after such injection is into the spinal and basilar subarachnoid spaces. The lampblack after a massive injection is found frequently in rather large amount in the temporal process of the lateral ventricle; in these cases the dilatation has not been great. The comparative freedom of the choroid plexuses from the granules, pointed out in kittens, has been noted in the adults.

Pathologically, the degree of dilatation of the lateral ventricles following subarachnoid injections of lampblack has been somewhat variable (cf. figs. 1 and 3).

A moderate injection of a 10 per cent suspension yielded in 5 days considerable ventricular enlargement (fig. 1), while the 5 per cent suspension gave in 16 days but little (fig. 3). Comparable subarachnoid injections of cinnabar and of lyco-podium spores caused no obvious abnormality in ventricular capacity (figs. 2 and 4).

#### DISCUSSION OF RESULTS.

The production of a typical internal hydrocephalus in kittens and adult cats by the injection of lampblack into the normal pathways of the cerebro-spinal fluid invites question as to the exact mechanism by which this enlargement of the ventricles is brought about. In both kittens and adult cats the condition has been caused by the injection of a suspension of these carbon granules through the occipito-atlantoid ligament into the cisterna cerebello-medullaris. The distribution of these granules subsequently is largely within the basilar and spinal subarachnoid space, with a smaller spread over the cerebral and cerebellar cortices. The maximal aggregation of granules is in the peribulbar cisterns with a minimal concentration within the ventricles. Following the intraventricular injection, as used in some of these kittens, the distribution of granules is largely intraventricular, basilar, and spinal, with but a small amount over the cerebrum. The same pathological picture in the main results from both types of injection.

After such experimental procedures there is usually a distinct interval of time between the initial injection and the appearance of signs indicative of intracranial pressure. In adult cats, this interval is usually of 6 or more hours. In very old cats, however, there seems to be really no time interval; the obstruction to flow seems complete enough to induce immediately a state of lethargy. This interval in kittens, however, is prolonged to 24 hours or more; usually enlargement of the head can be noted only after the fourth day.

It becomes necessary to reconcile, if possible, these somewhat divergent time-intervals and to attempt the formulation of an hypothesis to meet the conditions. The almost immediate appearance of a lethargy in very old cats indicates that at least a partial obstruction of the cerebro-spinal fluid is caused by the mere collection of these granules in the subarachnoid space. The fact that these older animals show symptoms immediately may be due to a decrease in the power of the medulla to resist alterations in pressure in the subarachnoid space. The increase in this pressure must be acute in all of the animals, but it seems likely that the obstruction to flow is only partial and that with a slightly increased head of pressure the fluid is forced through the incompletely occluded channels.

Subsequently, another factor seems to play a rôle and the blockage becomes apparently more complete. Two explanations for the formation of this obstruction may be offered: The first concerns the definite aggregation of the granules into a fairly impervious, impenetrable mass, mechanically hindering the flow of cerebro-spinal fluid. This probably plays a part in the initial blocking of the channels, but some other influence is doubtless necessary to render the aggregation of granules more impervious to fluid. This second factor is very likely the reactive phenomenon on the part of the body to the presence of such foreign granulation. Such a reaction

may be considered inflammatory, with more or less rapid deposition of fibrin and possibly the arousing of the fixed tissue-cells in the neighborhood. The reaction of the cells lining the subarachnoid spaces to the presence of carbon granules has already been noted (Weed, 1917). It may be assumed, then, that the block to flow of the cerebro-spinal fluid is dependent upon an aggregation of the granules into an impervious mass, the process probably being facilitated and the block perfected by the inflammatory reaction.

Question immediately arises as to the possible explanation of the failure of cinnabar and other granules, when injected in similar amounts into the cerebral ventricles and into the subarachnoid space, to give rise to internal hydrocephalus. This failure seems best explained by the assumption that these granules are not able to form as efficient aggregations even after the inflammatory reaction, so that the flow of the cerebro-spinal fluid is not impeded. This seems the more surprising for such a substance as lycopodium. With the cinnabar a minimal toxicity may modify the necessary inflammatory reaction.

It is quite difficult, after such injections of foreign particulate matter, to determine the exact point of obstruction to the flow of the cerebro-spinal fluid. The fact that similar pictures may be produced by intraventricular and by subarachnoid injections of lampblack indicates that in general a similar area of obstruction exists. Consideration of the density and distribution of the granules at autopsy inclines one to the view that the obstruction is primarily meningeal in character, and is probably a somewhat diffuse process in the basilar and possibly in the cortical portions of the subarachnoid space. The gross accumulation of granules occupies a portion of the cisterna cerebello-medullaris and the smaller cisterns of the base of the brain, but the mesh of the subarachnoid space is here of the maximum. To block this widened mesh completely seems more difficult than to do so in the cortical portion of the space, where the mesh is very fine and where a few granules and a reactive inflammatory process seem to have greater facilities for effecting a blockage of the channels for the cerebro-spinal fluid. It is very difficult to determine with certainty the ultimate obstruction to flow in these channels, but it must be considered as a diffuse intrameningeal process, whether in the basilar regions, or over the cortex, or about the various dural venous sinuses. The explanation, in these experimental animals as in man, for such obstruction to the flow of cerebro-spinal fluid through the meninges can not yet be given.

With the intrameningeal block of marked efficacy, the acute reduction of thickness of the cerebral cortex does not seem so remarkable. With such thinning to a very few millimeters, there is a possibility of two processes taking part. First, the thinning may be largely due to actual compression or destruction of brain-tissue. That this plays an important part in the enlargement of the ventricles in adults, where the cranial volume is fixed, must be granted; also, in cases of an infective hydrocephalus the two factors of compression and destruction work together. In the kittens, however, where the cranial capacity may be enlarged, the latter factor does not play the essential rôle. This is demonstrated by such findings as the retention (in the process of dilatation of the ventricles and thinning of the

cortex) of practically all of the essential intraventricular markings and structures; these are altered somewhat by the dilatation of the ventricles but remain easily identified. It must be granted, then, that the thinning of cerebral cortex in such internal hydrocephalus is the result largely of a rearrangement of tissues—a stretching and compression of the existing brain-tissue to cover the enlarging ventricles. In this enlargement some destruction of tissue may take place, but the essential process is a rearrangement of the bulk of cerebral tissue.

The obstruction to flow of the cerebro-spinal fluid, brought about by intraventricular or subarachnoid injection of lampblack, must be assumed, then, to be due to aggregation of these granules into an impervious mass, the essential and ultimate matting together being accomplished probably by an inflammatory process. Because of this obstruction, enlargement of the lateral ventricles follows, necessitating a diminution in thickness of the cortex cerebri, brought about by some tissue compression (as in adults) and by compression and readjustment of brain bulk (as in kittens).

#### SUMMARY.

An acute internal hydrocephalus may be produced by the intraventricular or subarachnoid injection of suitable amounts of a suspension of lampblack. In kittens the degree of this internal hydrocephalus is extreme, being associated with a marked enlargement of the head and other changes in the general appearance. This extreme dilatation of the ventricles with thinning of the cerebral cortex in kittens has been brought about in 10 days. In adult animals a similar dilatation of the lateral ventricles, but of lesser degree, may be caused by the same procedure. The internal hydrocephalus, produced experimentally in this way, is comparable in detail to similar conditions in man.



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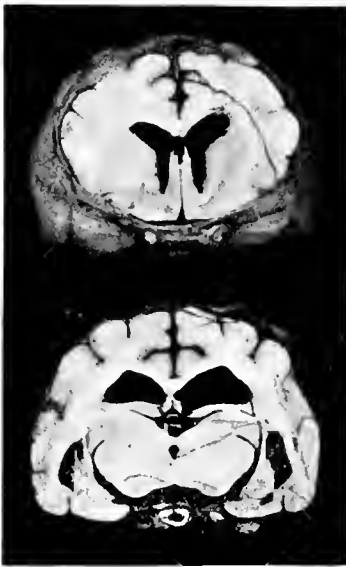
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## EXPLANATION OF FIGURES.

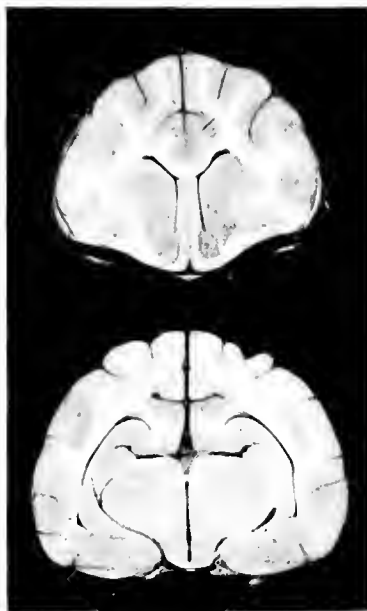
- FIG. 1. Photograph of two transverse sections of adult cat's brain (No. 90). The animal was given a subarachnoid injection of 5.0 c.c. of a 10 per cent suspension of lampblack in Ringer's solution and immediately afterwards exhibited signs of cortical excitation. Within 2 hours it became lethargic and could not be roused. It remained in this condition for 4 days and died on the morning of the fifth day. Considerable dilatation of lateral ventricles is shown.
- FIG. 2. Photograph of two transverse sections of adult cat's brain (No. 103). By occipito-atlantoid puncture, the animal was given a subarachnoid injection of 5.0 c.c. of a 10 per cent suspension of cinnabar in Ringer's solution. Animal remained normal and active, without sign of increase in intracranial pressure, for 8 days; it then developed distemper and died on the tenth day. No abnormalities in lateral ventricles are shown.
- FIG. 3. Photograph of two transverse sections of adult cat's brain (No. 99). Animal was given subarachnoid injection of 5.0 c.c. of a 5 per cent suspension of lampblack in Ringer's solution. Ten hours after animal went through stage of excitement, then became lethargic and slow; showed signs of a subacute increase in intracranial pressure and died on the sixteenth day. Full protocol in text. Slight enlargement of lateral ventricles is shown.
- FIG. 4. Photograph of a typical transverse section of the brain of an adult cat (No. 100). In this animal 5.0 c.c. of a 5 per cent suspension of lycopodium in Ringer's solution was injected into the subarachnoid space. Animal showed no signs of any increase in intracranial tension; it was normal and active. At end of 6 months it was sacrificed. The lateral ventricles appear as normally.
- FIG. 5. Photograph, during life, of a kitten receiving two intraventricular injections of 1.0 c.c. of a 5 per cent suspension of lampblack, 11 days apart. Following the first injection some ventricular dilation occurred, but after the second the process of enlargement was rapid. Photograph was taken on thirty-fourth day. Animal was sacrificed on the forty-sixth day after the first injection.
- FIG. 6. Photograph, taken immediately after death, of a kitten which when 9 days old was given intraventricular injection of 1.0 c.c. of a 10 per cent suspension of lampblack in normal salt solution. Animal lived for 14 days and was then sacrificed.
- FIG. 7. Photograph, taken immediately after death, of a kitten (Litter E<sub>2</sub>) which at age of 7 days received intraventricular injection of 1.0 c.c. of a 5 per cent suspension of lampblack in normal saline. After 15 days, received similar injection of 1.0 c.c. of a 10 per cent suspension; at this time it was noted that the fontanelles were opening. Subsequently the head enlarged rapidly and animal was sacrificed on the thirty-seventh day. Compare figure 6.
- FIG. 8. Photograph, taken immediately after death, of a kitten (Litter E<sub>2</sub>) which when 1, 7, and 22 days old received subarachnoid injections of 1.0 c.c. of 5 per cent suspension of cinnabar each. No abnormality in development or actions noted. Sacrificed on thirty-seventh day.

- FIG. 9. Lateral views of skulls of three kittens from same litter ( $F_2$ ). The animals were all sacrificed on the twenty-seventh day after the experimental procedure. The skull to the right is that of the control to which no injections were given. On the left is the skull of the kitten which received an intraventricular injection of 1.0 c.c. of a 5 per cent suspension of cinnabar. No change from the normal was noted in this kitten during life; at autopsy the skull was completely closed by bony union, as was the control's. In the center is the skull of the third kitten which received when 4 days old 1.0 c.c. of a 5 per cent suspension of lampblack. Typical internal hydrocephalus resulted.
- FIG. 10. Photograph of sectioned heads of same kittens as shown in figure 9. The skull-cap and underlying brain have been removed in same way from each. On the right is the control kitten; on the left is that of the kitten receiving intraventricular cinnabar. In the center is the hydrocephalic kitten which was given the intraventricular injection of lampblack.
- FIG. 11. Photograph during life of a kitten which received a subarachnoid injection of 1.0 c.c. of a 10 per cent suspension of lampblack, when 20 days old and when skull was closed. Photograph was taken on twelfth day after the injection. Full protocol of animal in text.
- FIG. 12. Lateral views of skulls of two kittens from same litter ( $N_3$ ). Above is the skull of the control animal while below is that of the same kitten as in figure 11. After receiving subarachnoid injection of lampblack, kitten lived 15 days; control was killed on same day.
- FIG. 13. Photograph of skulls of same kittens as given in figure 12. The control appears to the right, hydrocephalic on the left.
- FIG. 14. Photograph of sectioned heads of same kittens as recorded in figure 12. On the right is the control; on the left is the head of the kitten receiving subarachnoid injection of lampblack (cf. fig. 11).
- FIG. 15. Lateral view of skulls of two kittens from same litter ( $O_3$ ). On the right is the control; on the left is the skull of kitten receiving subarachnoid injection of 1.0 c.c. of a 10 per cent suspension of lampblack. At time of injection this kitten was 10 days old. Animal developed a typical hydrocephalus and was sacrificed with control on the twenty-second day after injection.
- FIG. 16. Photograph of sectioned heads of same kittens as in figure 15; on the right is the control, on the left is the hydrocephalic. The carbon granules occurring on the ventricular floor are typical of the deposit which occurs after such subarachnoid injection.
- FIG. 17. Photograph from above of skulls of two kittens from same litter ( $J_2$ ). On the right is that of the control animal, on the left is that of the experimental animal. This kitten when 12 days old was given a subarachnoid injection 1.0 c.c. of 5 per cent of lampblack in normal saline. The skull which was previously tightly closed opened up and wide suture lines were formed. Sacrificed, with control, on twenty-first day after injection.
- FIG. 18. Lateral view of same skulls as reproduced in figure 17. The control is below, the experimental hydrocephalic above.
- FIG. 19. Photograph of transversely sectioned skulls of same kittens as in figures 17 and 18; photograph taken from behind, looking toward nose. On the right is the control; on the left the experimental animal, showing resulting internal hydrocephalus.
- FIG. 20. Photograph of sectioned heads as in figure 19, taken looking toward the occiput. The enlargement of temporal cornua of lateral ventricles is shown.

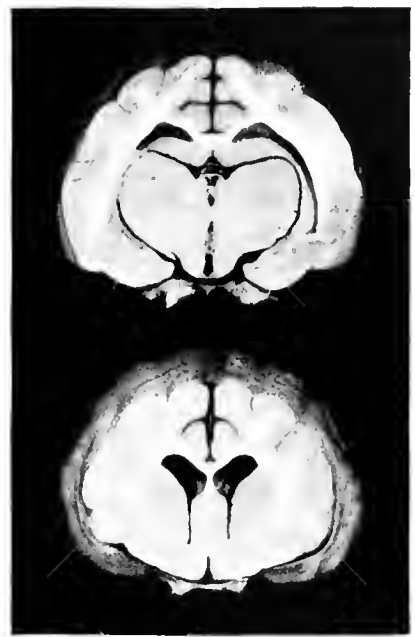
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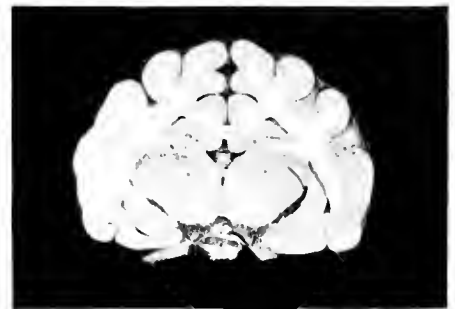
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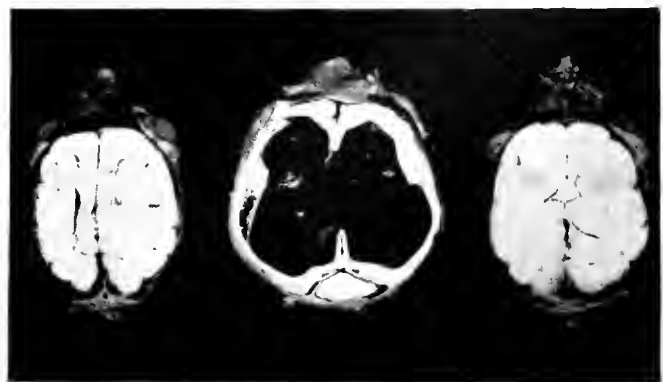
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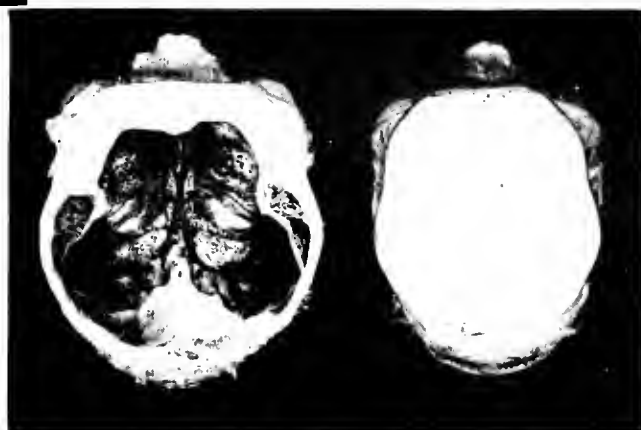
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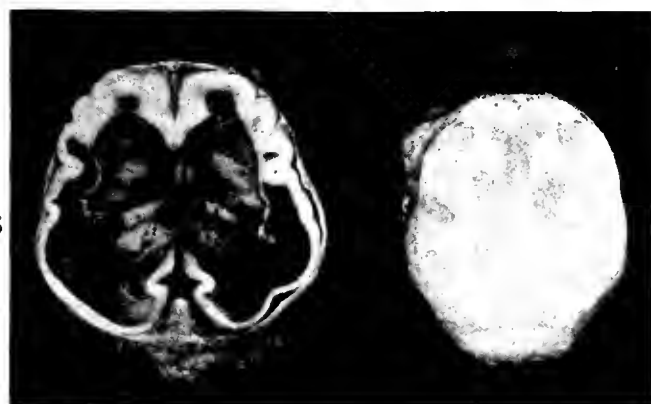
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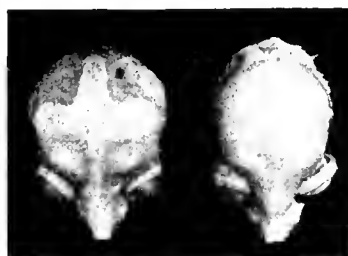
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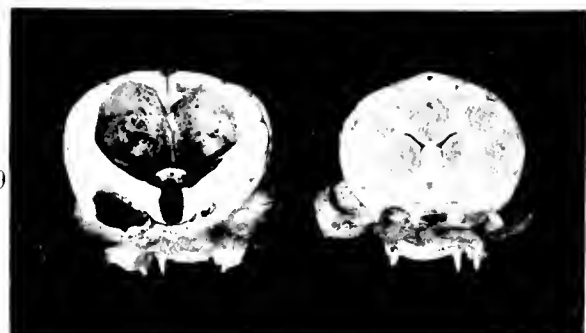
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CONTRIBUTIONS TO EMBRYOLOGY, No. 45.

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ON THE ORIGIN AND EARLY DEVELOPMENT OF THE LYMPHATIC  
SYSTEM OF THE CHICK.

BY ELIOT R. CLARK AND ELEANOR LINTON CLARK,  
*Of the Anatomical Laboratory, University of Missouri.*

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With seven plates and fifteen text-figures.

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## CONTENTS.

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	PAGE.
Introduction.....	449-450
History of earliest lymphatics in chick embryos.....	451
Method.....	453
Lymphatics of posterior lymph-heart region in chicks of 5 to 6 days.....	454
Lymphatics of posterior lymph-heart region in chicks of 4 to 5 days.....	460
Operations on chick embryos.....	465
Method of operation.....	466
Operation 1. Removal of posterior lymph-heart region.....	467
Operation 2. Removal of anterior point of origin for superficial lymphatics.....	469
Operation 3. Isolation of leg and pelvis.....	471
Operation 4. Isolation of anterior part of leg and pelvis.....	475
Summary of results obtained from operations on chick embryos.....	478
General conclusions.....	479
Bibliography.....	481
Description of plates.....	482



# ON THE ORIGIN AND EARLY DEVELOPMENT OF THE LYMPHATIC SYSTEM OF THE CHICK.

BY ELIOT R. CLARK AND ELEANOR LINTON CLARK.

These studies were begun and partly carried out in Professor Mall's laboratory in Baltimore, and have derived much from the inspiration of his enthusiastic and kindly interest. We believe it safe to say that few problems in anatomy were of as great interest to Professor Mall as that of the origin and mode of growth of the lymphatic system.

The numerous investigations and discoveries in this field during the past twenty years were started with the epoch-making studies of Sabin in 1901, begun at the instigation of Professor Mall and, almost without exception, can be attributed indirectly to his guiding genius. It is an interesting illustration of his method of achieving results that not one of the papers on the development of the lymphatic system, published from his laboratory during this period, carries his name on the title page; although, he might, with justification, have insisted that many of these publications include his name as joint author. That he did not is evidence of his pure unselfishness and his realization of the importance of developing early the sense of independence, responsibility, and achievement in his students, if they, in turn, were to become sturdy, independent anatomists.

It is surprising to review the many changes in and additions to our knowledge of the lymphatic system which have taken place since 1900, when Professor Sabin began her studies. Previously, the conception of adult anatomy of the lymphatic system was in a very hazy state. In fact, with the exception of the lymph-glands, the thoracic duct, and other large lymphatics, it could scarcely be designated as a real *system*, since it was supposed to merge, in obscure and devious ways, with the peritoneal cavity, with the serous cavities in general, and with "spaces" everywhere in the tissues. Even the discovery of von Recklinghausen (1862) that silver nitrate stained the endothelium of lymph-vessels in a definite manner was obscured by the faulty interpretation, which explained these silver markings as revealing a sieve-like structure of the lymphatic wall, whereas they are now known to represent cell-boundaries in a definite membrane. As for the embryology of the lymphatic system, it was practically a virgin wilderness lighted only by the uncertain torch of Budge's work, in which his injection of the extra-embryonic coelom was interpreted as a picture of the primitive lymphatic system.

To-day, less than twenty years after the beginning of the investigations in Dr. Mall's laboratory—years fraught with strenuous controversy at times presenting seemingly irreconcilable views—we possess an adequate knowledge of the structure

of the lymphatic system in the adult; a definite idea of the manner of growth of the lymphatic capillary and a few hints as to some of its reactive powers; a picture of the development of some of the component parts of the system, such as the ducts, sacs, and lymph-hearts, and, in some instances, a correlation of their morphology with their function; a history of its development in many organs of the mammalian embryo and of its differences in pattern and extent in various animals; and a fairly complete idea of its connections with the venous system and of the persistence of such communications in the adult. A more and more complete picture of a definite, independent, and important system has emerged from all these studies, together with a history of the manner in which this system has developed from the earliest beginnings—where, however, the outlines of the picture once more become hazy. Since most of this work has been recently reviewed by Sabin (1913 and 1916), it seems unnecessary to enter into a detailed discussion of literature. The work done since that time, and those investigations which bear directly upon the particular problems considered here, will be referred to in the course of the descriptions.

In the present investigations we have attempted to carry the study back to the first appearance of the lymphatics in the embryo, since this part of the history seemed to be still lacking in clearness. The study involved the consideration of the following unsolved problems: (1) The nature of the earliest lymphatics. (2) The tissue from which they originate. (3) The manner in which the differentiation is accomplished. (4) The question as to how soon the lymphatics form a specific, independent system, whose growth proceeds by the sprouting of pre-existing endothelium in the manner proved for the stage of development of the vessels in the tail of the frog larva (Clark, 1909). (5) The question of the points of origin of lymphatic endothelium in the embryo; whether there are strictly limited areas in which the first lymphatic endothelium arises, or whether the differentiation is diffuse.

For convenience the description of these investigations and the results obtained have been divided into two parts. Part I deals with the history of the earliest lymphatics in chick embryos and is concerned with the problems of their character and origin. Part II is a report of the operations on chick embryos and is concerned with the question of the points of origin of the first lymphatics.

## I. THE HISTORY OF THE EARLIEST LYMPHATICS IN CHICK EMBRYOS.

In approaching this phase of the problem we decided to select some one region of the embryo and to study it intensively by all available methods, assuming that the manner of formation of the earliest lymphatics follows the same laws in other regions. The posterior lymph-heart region was chosen because of its accessibility for observation in the living and for direct injection, and because previous work had made us familiar with it.

The posterior lymph-heart of the chick was discovered in 1882 by Budge, and various stages in its development have since been described by Sala (1900), Mierzejewski (1911), E. R. and E. L. Clark (1912), and West (1915). The region of the posterior lymph-heart is situated on either side of the tail, beginning at the angle formed by the posterior border of the pelvis and the tail, and superficial and lateral to the myotomes. In chicks of 8 days the lymph-hearts in this region are clear, almond-shaped bodies, easily visible in the living embryo (as stated by Sala and by Budge), and their pulsations can be noted. Sala found that the lymph-hearts communicate with the intersegmental coccygeal veins, connecting at first with five of these veins and later with three. By the tenth day the hearts are rounded in form and surrounded by a layer of fat. Budge injected the lymph-hearts from the allantoic lymph-vessels in chicks of 9 days. He found that they were present in the chick until after the time of hatching, but were absent in adult chickens. Stannius (1843) and Panizza (1830) had previously shown that lymph-hearts are present in adult aquatic birds as well as in the ostrich and cassowary.

In studying earlier stages of the lymph-heart Sala made use of cross sections of chicks of  $6\frac{1}{2}$  to 7 days old. In these he describes a series of "spaces" connected with the intersegmental coccygeal veins and concludes that the lymph-heart is formed by the coalescence of these spaces; at one time he refers to them as mesenchymal spaces and at another as continuations of the veins themselves.

In 1901 Sabin published her paper describing the embryonic lymphatic system of the pig, which, in the earliest stages, consisted of paired jugular and iliac sacs and the thoracic duct connecting them. These sacs were connected with veins and seemed to be the homologues of the lymph-hearts of lower animals, and Sabin concluded at the time that they were outgrowths from the veins and were the primary points of development for the lymphatic system, from which all other vessels were derived by a process of outgrowth. This view that the lymphatics grow by sprouting had been advocated by Ranvier (1895) from studies made on much older embryos.

In 1906 F. T. Lewis carried the history of the lymphatic system back a step farther by his studies of rabbit embryos. In the region of the jugular lymph-sac, and before the appearance of that structure, he found a discontinuous plexus of capillaries lined with endothelium. From the study of fixed material he suggested the possibility that these were "veno-lymphatics" or blood-vessels, formerly part of the general circulatory system, which had become cut off and were about to be transformed into a lymph-sac. Huntington and McClure (1910) described a stage

of veno-lymphatic and pre-lymphatic vessels as preceding the appearance of the jugular sac in cat embryos, and Miller (1912) figured a similar discontinuous pre-lymphatic plexus in the region of the jugular lymph-sac of chicks.

In 1909 Mierzejewski studied the superficial lymphatics of chick embryos by the injection method of Hoyer, and showed that there is a plexus of capillaries present in the region of the posterior lymph-heart in chicks of  $5\frac{1}{2}$  days, 24 hours earlier than the first appearance of the lymph-heart rudiment, as described by Sala. Mierzejewski considered this to be a lymphatic plexus which had grown out from the coecygeal veins.

In 1911 we began our studies of this region in chick embryos and portions of these have been published from time to time. We observed that the lymph-heart begins to pulsate in chicks of 6 to  $6\frac{1}{2}$  days, and that the early contractions occur only at the time of the periodic movements of the embryo. When granules of India ink were injected into this translucent, pulsating area, a rich plexus of lymphatic capillaries was revealed, thus showing that the lymph-heart of the chick is a functioning heart before it is a sac (Clark and Clark, 1912, 1914). Similarly, a continuous plexus of lymphatic capillaries was injected in the jugular region at a stage in which Miller had found the discontinuous "pre-lymphatic" anlage (E. L. Clark, 1912).

While studying this posterior lymph-heart region in living chicks of 5 to 6 days, a stage before a beating lymph-heart is present, we found in this and adjacent areas a richly anastomosing plexus of capillaries, independent of the surrounding blood-capillaries and easily distinguishable from them, chiefly by means of the stagnant blood which they normally contained. Preliminary reports of studies of these early lymphatics, made on living chicks, have been published (Clark and Clark, 1912). The discovery that early lymphatics contain stagnant blood enabled us to directly inject lymphatic capillaries with much greater facility and precision than before, and by this method to study these early vessels and their relation to the blood-vascular system (E. L. Clark, 1912*b*). A preliminary report of microscopic studies of these early lymphatics has also been published (E. R. Clark, 1914), in which it is stated that the early lymphatic endothelium possesses definite morphological characteristics which distinguish it from the mesenchyme cells.

In later studies made on pig embryos Sabin (1912, 1913) reported the finding of lymph-capillaries, filled with stagnant blood, which were connected with the jugular vein and with the abdominal veins in stages before sacs are present in these regions. Also, West (1915) published a study of the posterior lymph-heart region in the chick, in cross-sections of which he found a continuous plexus of lymphatic capillaries before the formation of a lymph-heart.

These various studies apparently demonstrate the incorrectness of the view that lymph-sacs and lymph-hearts are the primary structures of the lymphatic system, and also that they are derived from veno-lymphatics which at one time functioned as blood-vessels containing circulating blood. They point instead to the existence of a continuous plexus of lymphatic capillaries which subsequently becomes converted into lymph-hearts and lymph-sacs.

Although the origin of this primary lymphatic plexus has not yet been determined for birds and mammals, studies have been made on what appear to be the first stages of lymphatic development in amphibians. Fedorowicz (1913) studied the origin of the posterior lymph-heart in frogs, and described the first lymphatic vessels as solid strands of cells derived from the endothelium of the lateral caudal vein. These subsequently acquire lumina, unite with one another, and later coalesce to form the lymph-heart.

Kampmeier (1915) studied the early stages in the development of the anterior lymph sinus in *Bufo*. His discovery that the endothelial cells in this space retain the yolk-granules longer than do the connective-tissue cells made possible a careful cytological study of the early stages with high powers of the microscope. Kampmeier came to the conclusion that the first lymphatic vessels are derived from the veins by a process of outgrowth in a number of places. These outgrowths, many of which are at first solid, unite with one another to form the plexus which precedes the formation of the lymph-sinus.

Beginning with this work in 1912, the authors have spent several years in a careful study, by various methods, of these early lymphatic capillaries in the region of the posterior lymph-heart of the chick in an effort to discover their origin.

#### METHOD.

Our method of studying living chicks has previously been described (Clark and Clark, 1912, 1914). The essentials are a binocular microscope inclosed in a warm chamber kept at incubator temperature, Ringer's solution for keeping the chick moist, and a bright light for direct illumination (sunlight or a desk-lamp). A large window was made in the egg-shell, and the amnion opened and pulled aside. In this way chicks were kept alive and active for 7 or 8 hours.

For the lymphatic injections we used India ink, diluted to one-half with tap water, and fine glass cannulae (10 to 15  $\mu$  at the tip) attached to a rubber tube. For double injections we used various materials, India ink for the lymphatics and Berlin blue with 5 per cent gelatin for the blood-vessels; or India ink in the blood-vessels and Berlin blue in the lymphatics. We also injected lymphatics with silver nitrate (0.5 per cent) and the blood-vessels with India ink. Such injected specimens were fixed in Carnoy's fluid, dehydrated in absolute alcohol, and cleared in benzol and oil of wintergreen. Or, if we desired to section such embryos, they were fixed in Bouin's fluid.

For our studies of sections the blood-vessels were injected completely with India ink. Good injections were obtained either through the allantoic artery or through one of the vitelline veins. In either case the heart was allowed to pump the injection mass around through the body. The umbilical cord was tied and the embryo taken from the yolk and dropped into the fixing solution. For this purpose we obtained the best results by the use of McClung's modification of Bouin's fluid: Saturated aqueous solution of picric acid, 75 per cent; formalin (40%), 20 per cent; glacial acetic, 5 per cent.

The embryos were carefully dehydrated to avoid shrinkage, and then embedded in paraffin. In some cases cross-sections of the tail region were made, but in most

instances the sections were cut *parallel to the surface*. By this means the greater part of the lymphatic plexus was contained in a few sections and parts could be reconstructed which would be completely lost in cross-sections. The sections were cut 10 and 15  $\mu$  in thickness, stained on the slide with Ehrlich's hematoxylin, and counterstained with a mixture of eosin, orange G, and aurantia. By this staining method the nucleoli of the lymphatic endothelial cells are reddish in color, which is an aid in distinguishing them from the mesenchyme cells whose nucleoli are bluish-purple. This same contrast was obtained by the use of Mann's methyl-blue eosin stain.

Such sections were studied by means of oil-immersion reconstruction. The blood-vessels could be identified by the presence of India ink, and the other vessels were all carefully drawn, every strand of endothelium, every nucleus and nucleolus being recorded. The structures were not studied in this manner until extensive investigations of the lymphatics and blood-vessels of this region had been carried out in living and injected embryos. Familiarity with the region as a preparation, and the modification of parallel sections, the constant use of the oil-immersion lens, and a criterion for distinguishing endothelial cells enabled us to detect definite vessels and even a plexus at stages in which our earlier studies and those of other investigators gave no hint of the existence of lymphatics. Needless to say this method is extremely tedious. A drawing of all the endothelial cells, including nuclei and nucleoli, was made of each section, and the drawings of successive sections were combined in graphic paper reconstructions.

#### LYMPHATICS OF POSTERIOR LYMPH-HEART REGION IN CHICKS OF 5 TO 6 DAYS

As has been stated, the pulsation of the posterior lymph-heart can be seen in chicks of 6 to 6½ days, a stage when injection shows that the heart is still in the form of a plexus. This lymph-heart plexus connects medially with the first five intersegmental coccygeal veins, and ventrally with a superficial lymphatic plexus which spreads out over the pelvis and anterior body-wall, and is continuous from the tail to the axilla. Cleared, injected specimens show that this superficial plexus has connections through the axillary region with the deep lymphatic plexus located dorsal to the anterior and posterior cardinal veins, near their junction at the duct of Cuvier, with which veins it communicates at a number of places. (A drawing of this plexus has been published in an earlier article—E. L. Clark, 1912.) In a chick of 5 days and 18 hours this continuous superficial plexus can be injected and most of it can be seen in the living chick because of the stagnant blood present in its lumen. The lymph-heart has not started to beat at this stage.

In younger embryos (5 to 5½ days) blood-filled lymphatics are also visible, but a continuous plexus over the surface of the body can not be seen in the living or injected specimens. Instead, a plexus is found anteriorly, near the region of the thoraco-epigastric vein, and connected through the axillary region with the deep jugular plexus and with the veins, as in the older specimen; also another posterior plexus connected with the intersegmental coccygeal veins and confined to the region of the posterior lymph-heart and to the neighboring area over the posterior

tip of the pelvis. In these younger embryos the lymphatic plexus, as seen in the living or injected specimens, is less luxuriant than in the chicks of  $5\frac{1}{2}$  to 6 days, and the vessels composing it are much finer.

The appearance of the posterior blood-filled lymphatic plexus in the living chick is illustrated in plate 1, figure 17. The more superficial portion, over the tip of the pelvis, connects with the deeper plexus which later forms the lymph-heart. This superficial plexus is easily distinguishable from the blood-capillaries of the region in a number of ways. For the most part it lies beneath the superficial blood-capillaries. The pattern of the two sets of vessels is different; the lymphatic capillaries are more irregular in form than the blood-vessels. The dark-red color of the plexus containing stagnant blood contrasts with the lighter, more yellowish tinge of the blood-capillaries. The most striking feature is the contrast between the rapid motion of the blood-corpuscles in the blood-capillaries and the blood in the lymphatic plexus which remains stagnant. Observation of the two plexuses shows clearly that this plexus of capillaries filled with stagnant blood is a distinct and independent system of vessels.

Various tests were also made in order to learn more of the character of this early plexus and its relation to the blood vascular system. The plexus containing stagnant blood was injected by direct puncture of a selected lymphatic capillary. The near-by circulating blood-vessels remained undisturbed after such an injection, thus showing the independence of the two systems. Figure 21, plate 2, illustrates such an injection in the exact location occupied by the beating lymph-heart of older embryos and a few delicate superficial vessels continuous with it. The injection often reveals a few fine connections which had not been observed in the living, but in general the extent of the plexus, as demonstrated by the presence of the blood and by injection, is the same.

When a small amount of ink was injected into one of the vessels of this lymph-heart plexus without disturbing any of the superficial blood-capillaries, the granules could be seen to enter the intersegmental coccygeal veins and to move along in the main caudal vein. Cleared specimens with injected lymphatics enabled us to study this relationship of the veins still further. Figure 1 shows the lymph-heart plexus and its venous connections of both sides. The view is a dorsal one, for with the chick in its natural position, lying on one side, the lymph-heart plexus lies directly over the intersegmental veins of the tail, thus concealing the points of connection from the observer.

Complete blood-vessel injections were obtained, and such an injection left the lymphatic plexus filled with stagnant blood, while all the surrounding blood-capillaries became filled with the injection mass. Figure 18, plate 1, is a drawing of a fresh specimen made immediately after such a blood-vessel injection.

Double injections were obtained with the blood-vessels completely injected with India ink and the lymphatics filled with silver nitrate or Berlin blue. Plate 3, figure 24, shows such an embryo with some early lymphatics in the lymph-heart region injected with silver nitrate. A thick cross-section made from the same specimen (plate 3, fig. 25) shows the location of some of these little vessels with

regard to one of the intersegmental coccygeal veins and to the superficial blood-capillaries. When this blood-filled plexus was injected directly with silver nitrate (0.5 per cent) the characteristic endothelial markings appeared, demonstrating that these vessels can not be simple mesenchymal spaces, as might be inferred from Sala's descriptions.

When the large arteries and veins of the yolk-sac and allantois were opened and the embryo was allowed to bleed freely, the blood could be seen to fade out of the superficial blood-capillaries, but the lymphatic plexus remained undisturbed and still filled with stagnant blood. An embryo thus bled makes a rather striking picture, with the bright-red lymphatic plexus standing out against the white background. Figure 22, plate 2, illustrates the superficial lymphatic plexus in a 6-day chick that had been bled in this manner.

Examination of the superficial plexus in sections of chicks of 5 to 6 days in which the blood-vessels had been completely injected confirmed the observations, made by the other methods, that there is a continuous plexus which is independent of the surrounding blood-vessels except for connections with branches of the intersegmental coccygeal veins. In addition, the sections showed that this plexus possesses a definite endothelial lining, and that the endothelial nuclei of the lymphatics have certain morphological characteristics which distinguish them from the nuclei of the adjacent connective-tissue cells. These nuclei have a pale, fairly homogeneous, granular appearance, and contain a single nucleolus or a pair of nucleoli, which are definite, discoid bodies, sharply marked off from the remainder of the nuclear material by clear-cut, rounded outlines. The definiteness of this endothelial nucleolus was shown in a striking manner in cases in which an endothelial nucleus had been cut into during the process of sectioning. In many such instances the nucleolus had been dragged out of the nucleus but still retained its characteristic shape. The single nucleolus varies in form according to the shape of the nucleus and to the plane in which the cell has been cut. With the method of staining used these nucleoli have a distinctly *reddish* color.

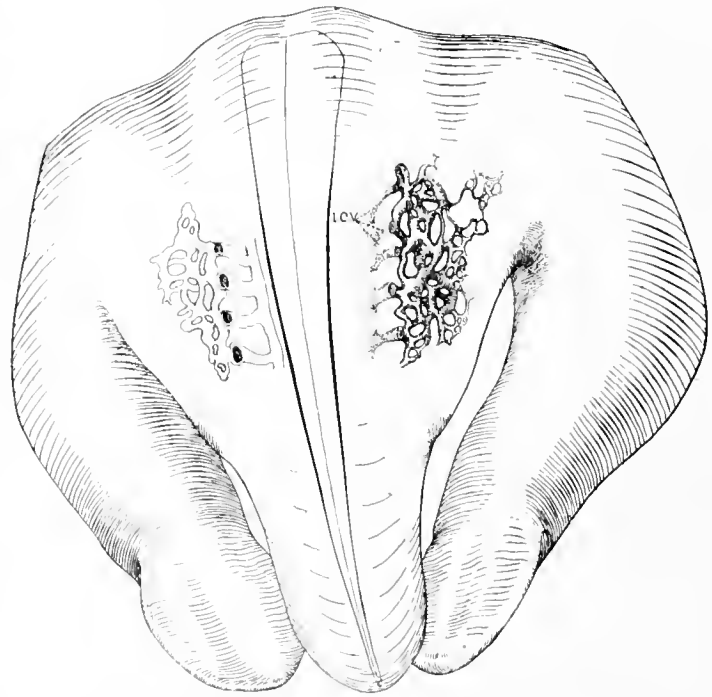


FIG. 1.—Dorsal view of tail region of an injected embryo of 5 days 23 hours, showing the lymph-heart plexuses on both sides and the coccygeal veins with which they are connected. *i.c.v.*—intersegmental coccygeal vein.  $\times 24$ .



The nucleus of the mesenchyme cell differs in all the particulars mentioned. It contains two or more nucleoli which are not sharply differentiated from the remainder of the chromatin material of the nucleus, but which extend out into prongs and threads, and these do not have a characteristic shape. These nucleoli take a distinctly *bluish* stain. The remainder of the nucleus of the connective-tissue cells is darker in appearance than that of the endothelial cells and frequently contains small clumps of chromatin material. In chicks of this stage the blood-vessel endothelial nuclei are slightly smaller than those of the lymphatics, more regular in shape, and are apt to contain two nucleoli instead of a single large one. In earlier stages these distinctions are frequently absent, and without the injection material present in the blood capillaries it would be practically impossible to distinguish the two types of nuclei. Figures 26 and 27, plate 4, show microscopic drawings of a younger embryo (4 days and 23 hours) and illustrate the difference between the endothelial nucleus and the nucleus of the mesenchyme cell.

An examination of sections confirmed the observations based upon injections and the study of the living; *i. e.*, that in chicks of 5 to 6 days a primitive plexus of lymphatics is present in this posterior part of the pelvis and in the tail region and precedes the formation of the lymph-ducts and the beating lymph-heart which occupy the same area in older embryos. The plexus is indifferent and irregular in character; comparatively large bulbous nodes alternate with very fine, even solid connections and delicate processes. The connections of this plexus with the first five intersegmental coccygeal veins or their branches were easily seen in sections, but aside from these the plexus was found to be independent of the blood vascular system. A number of mitotic figures were seen in the endothelium of this lymphatic plexus. The presence of these mitoses, scattered here and there in endothelial cells, as well as the general character of the plexus, gives the impression of a wild and rapid growth.

In studying sections, cut parallel to the surface, of chicks of 5 to 5½ days in which the blood-vessels had been completely injected, with the aid of the oil-immersion lens a more extensive plexus of lymphatics could be made out than we had been able to discover with other methods. For example, figure 3 is from a reconstruction of a portion of the plexus over the pelvis of a chick of 5 days 7½ hours. In this specimen the blood-filled lymphatics of the posterior part of the pelvis and of the lymph-heart region were injected with India ink and the blood-vessels subsequently filled with Berlin blue gelatin (5 per cent). The drawing shows a part of the rich plexus of fine, delicate vessels which we were able to reconstruct in an area well beyond the injected part. The reason why the injection failed to reveal this is obvious from the character of the plexus. Although the endothelium is continuous the lumen is not. Many of the connections are exceedingly narrow and others are entirely solid. It is interesting to note the large number of mitoses present in this small part of the plexus.

As we stated in 1912 and 1915, the stagnant blood normally present in the early lymphatics of this stage is due merely to pressure conditions. It is easy to show by injection experiments on living chicks, by cleared injected specimens, and

by microscopic sections, that the early lymphatic system in chicks of this stage is connected in certain regions with the venous system. No valves are present at this time. Although there is evidence from study of parallel sections that an absorptive function has already started in these vessels, such function is comparatively slight and not sufficient to overcome the lateral pressure of the veins. That the blood enters the lymphatic system from the veins can be demonstrated in a number of ways.

(1) The amount of blood present in the superficial lymphatic system can be increased in the living chick by changing the position of the embryo so as to allow the force of gravity to affect its various parts. For example, the posterior lymphatics,

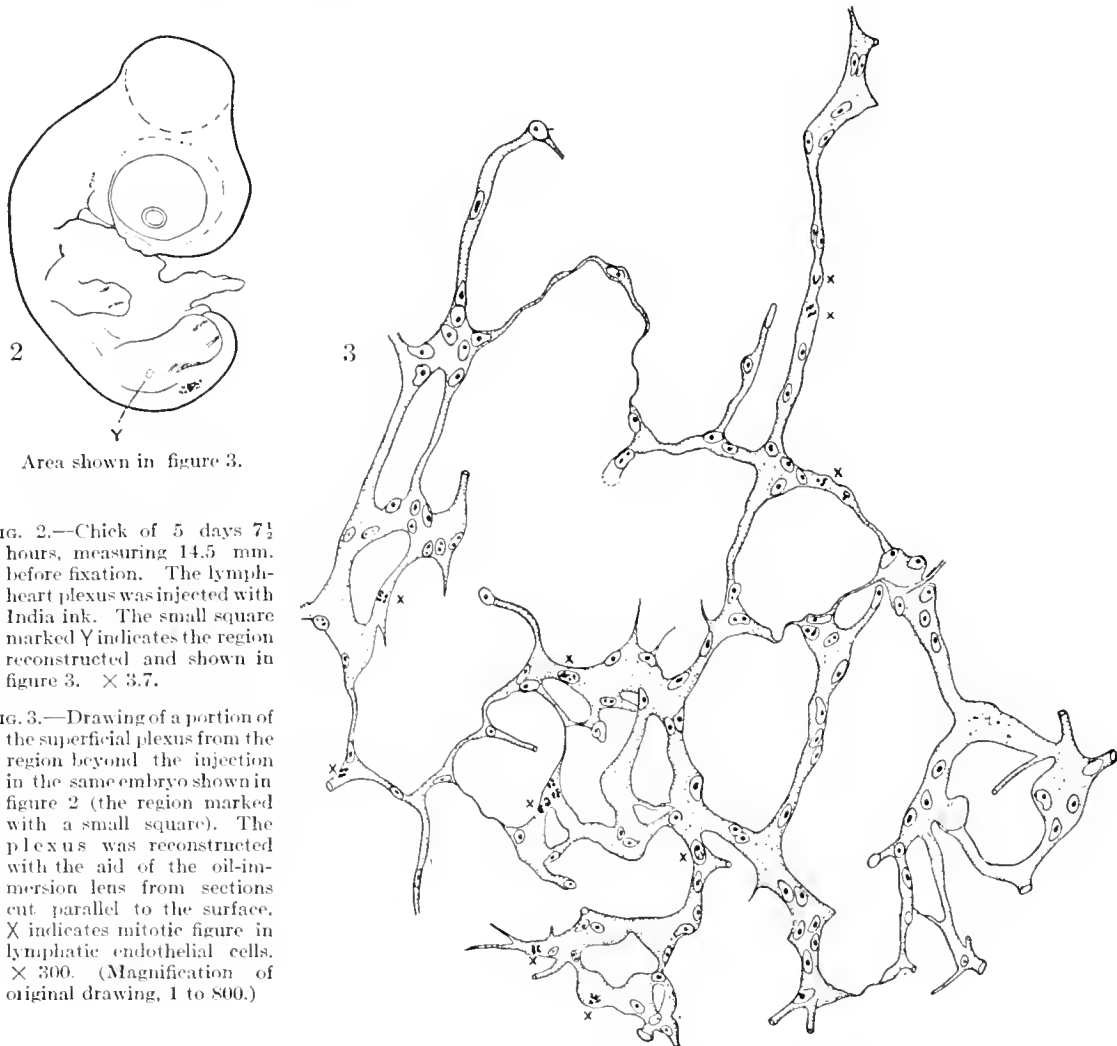


FIG. 2.—Chick of 5 days 7½ hours, measuring 14.5 mm. before fixation. The lymph-heart plexus was injected with India ink. The small square marked Y indicates the region reconstructed and shown in figure 3.  $\times 3.7$ .

FIG. 3.—Drawing of a portion of the superficial plexus from the region beyond the injection in the same embryo shown in figure 2 (the region marked with a small square). The plexus was reconstructed with the aid of the oil-immersion lens from sections cut parallel to the surface. X indicates mitotic figure in lymphatic endothelial cells.  $\times 300$ . (Magnification of original drawing, 1 to 800.)

phatics, which spread out over the surface of the tail and over the posterior border of the pelvis, lie, for the most part, anterior to the coccygeal veins with which they are connected. By pulling on shreds of the amnion the tail can be raised so that this region is uppermost, and the embryo can be held in this position for some min-

utes, at the expiration of which more blood enters the lymphatics and the anterior (lower) portions of the plexus become much redder and more distended.

(2) Again, the amount of blood in the early lymphatic plexus can be increased noticeably and quickly by interfering with the blood circulation. When the heart becomes embarrassed from any cause (such as the addition of strong chloretone or too high temperature of the warm chamber) there occurs a back pulsation in the veins, which can be observed to the best advantage in the large vessels of the allantois and yolk-sac. Within a few seconds after the beginning of such a circulatory disturbance the superficial lymphatics become markedly redder and more congested, owing to the increased amount of blood which enters them as a result of this increased venous pressure.

(3) Further evidence for this backing up of the blood from the veins was found in our studies (1915) of the beginning lymph flow in chicks of 6 to 6½ days. In these we traced the steps in the process by which the blood is washed out of the lymphatics. In the stages of its first pulsation, while the flow is relatively feeble, the lymph-heart was observed to fill up with blood in the interval between beats. These observations also showed that there is a stage in the development of the superficial lymphatics in which the pressure in the lymphatics overcomes that of the veins.

(4) In chicks of 7 days, a stage in which there is normally no stagnant blood present in the lymphatics, it was found that the flow of lymph is more rapid and the lymph-heart contractions are stronger. If the action of the lymph-heart is paralyzed by chloretone, a drug which also increases slightly the back pressure in the veins, the lymph-heart and adjoining vessels soon fill up with stagnant blood and become visible once more in the living embryo.

(5) The pictures obtained from sections of chicks of 5 days also bear out this point, for the newer portions of the plexus (those portions which do not possess a continuous lumen) are quite empty of blood-cells, and hence invisible in the living; and the blood is present only in the portion next to the veins where the lumen is continuous.

All these observations, therefore, show that the stagnant blood present in the lymphatic plexus of chicks of 5 to 6 days has backed up from the veins. Its presence is merely a transitory pressure phenomenon.

Some writers have claimed that chick lymphatics have a "hemophoric" function, meaning that they carry newly-formed blood-cells from the tissues to the blood-vessels (Miller, 1913; West, 1915). That lymph-capillaries have the power of picking up blood-cells that have become extruded into the tissues was shown by one of the authors in 1909. In sections of some of the earlier chicks of this series (4 to 5 days) peculiar, large cells were found to be present in the mesenchyme, which might be interpreted as blood-forming cells or as large phagocytes; but for the stages in which the blood-cells are present in such quantity as to render a large plexus visible under the binocular microscope or even to the naked eye, all the evidence points to the backing-up of this blood from the veins as the true interpretation.

## LYMPHATICS OF POSTERIOR LYMPH-HEART REGION IN CHICKS OF 4 TO 5 DAYS.

As stated above, the plexus of blood-filled lymphatics in the region of the posterior lymph-heart, and in the more superficial regions ventral and anterior to it, is easily visible in living chicks of  $5\frac{1}{2}$  days. Pushing the study of this region back to still earlier stages, we found the first evidence of lymphatics in the living in embryos of about 12 mm. greatest length (measured in the fresh) and about 4 days 20 hours to 5 days old. These first lymphatics were visible as a number of separate knobs of stagnant blood in the region just lateral to several of the dorsal intersegmental coccygeal veins. At this stage these little dots of blood give the region of the posterior lymph-heart a characteristic speckled appearance. Since the knobs lie between the observer and the veins their connections can not be seen, but ink granules injected directly into them can be observed to pass into the nearest intersegmental coccygeal vein.

In many cases we studied this region in the living 4 to 8 hours before the appearance of these knobs, and were satisfied that no circulating-blood vessels are present in the exact area where these vessels make their appearance. About an hour after the first knobs become visible new ones can be seen near them, and connected by narrow vessels. Injection of these structures shows small, discrete clusters, somewhat resembling bunches of grapes, connected, as were the earlier knobs, with the intersegmental veins of the tail. The steady and rapid extension of these blood-filled structures to form a plexus was observed in many embryos. They extend toward the surface and spread out in the region superficial and ventral to the lymph-heart region. Two or three delicate projections from the plexus can first be seen, connections between these make their appearance, then certain parts of the plexus enlarge and become more densely packed with blood-cells. New sprouts appear in advance, and the same process of extension, accompanied by plexus formation, is repeated. The several parts of the plexus are irregular in size; many of the lymphatics are several times as wide as a blood-capillary, while some of the connections and processes are as small as and often much smaller than a blood-capillary. All of these successive stages were tested by pressure over the parts filled with blood, in order to see if the blood could be forced farther, and also tested by injection. By both of these methods it was found that, in these early stages, practically all of the injectible lymphatics are normally filled with stagnant blood. This blood, which backs up from the veins with which the early lymphatics connect, forms a vital injection mass which is constantly being forced into the developing plexus, and by this means reveals the extension of the continuous lumen of the lymphatics.

As already stated, in chicks of 5 days, sectioned parallel to the surface, a plexus of delicate vessels could be reconstructed, by use of oil-immersion, well beyond the limit of the injectible or blood-filled lymphatics. We next studied still younger embryos by this method of paper reconstruction, with the aid of the oil-immersion, in an effort to trace back the picture of the developing lymphatics.

Plate 5 is a reconstruction of the region of the posterior lymph-heart in a chick of 4 days 23 hours, measuring 12 mm. in the fresh state. In this specimen, examined in the living, the first knobs filled with blood were visible. They were not injected, but from other specimens of the same stage it may safely be inferred that injections would have shown us small clusters connected with the intersegmental coccygeal

veins. When the sections of the embryo were studied with the higher magnifications a continuous plexus was reconstructed in this region. Some of the vessels are as large as blood-capillaries and contain blood-cells; these undoubtedly represent the blood-filled knobs seen in the living; others are much narrower, and still others are solid. Long narrow processes extend superficially and ventrally from this plexus in the exact position in which the blood-filled sprouts make their appearance in living chicks a few hours later. The plexus connects at several points with branches of the intersegmental coccygeal veins in each segment (X in the drawing). Some of these connections are fairly large and others are solid.

This lymphatic plexus is composed at this early stage of continuous endothelium which has the characteristic endothelial nuclei already described. Figures 26 and 27, plate 4, were drawn from vessels composing this same plexus and show the differences in form and color between the nuclei and their nucleoli and those of adjacent connective-tissue cells. Figure 29, plate 4, is also from this plexus and shows a lymphatic endothelial cell in mitosis. Figure 28, plate 4, is from another specimen of the same stage and shows one of the early blood-filled knobs in section, with its connections with the veins. One of these connections is open and contains a blood-cell, while the other is solid and thread-like.

Just as the study of the living blood-filled lymphatic plexus in chicks of 5 to 6 days showed that the reconstruction from cross-sections gave only a very incomplete account of the true conditions (F. T. Lewis, 1906, and Miller, 1912), so the picture obtained from these studies, made with the oil-immersion, shows that our earlier account of the blood-filled "bunches" seen in the living and injected at this stage (Clark and Clark, 1912) was extremely fragmentary. This is also the stage which West (1915) describes as his first stage (a chick of the same age as this specimen, and one measuring 10.5 mm. after fixation and dehydration), in which only a few "mesenchymal spaces" could be reconstructed in this region. Figure 31, plate 6, represents the condition in a part of the region in a still younger embryo. This chick was 4 days and 9 hours old and measured 11.75 mm. in the fresh state. No blood-filled lymphatics could be seen in the living chick. The blood-vessels were completely injected with India ink and the embryo fixed, sectioned, and stained in the usual manner. The oil-immersion reconstruction showed us that even at this stage a plexus of vessels, with the characteristic form of an early lymphatic plexus and the characteristic endothelial nuclei, is present in the same region which is later occupied by the blood-filled lymphatic plexus, and still later by the beating lymph-heart. The plexus at this stage is not so luxuriant as that of the next older stage shown in figure 30, plate 5, and the vessels composing it are relatively smaller. The fine and solid connections between different parts of the plexus are relatively more numerous. A number of venous connections are also present at this early stage. Finally, figure 32, plate 6, is taken from an oil-immersion reconstruction of a portion of this region in a chick of 4 days and 7 hours, measuring 9 mm. in the living. Here, besides the injected blood-vessels and the indifferent mesenchyme, there were suspicious-looking vessels with characteristic endothelial nuclei in a part of the region chosen for intensive study. As in the other sectioned specimens, all of these nuclei were drawn, with the characteristic nucleoli, as was also the endo-

thelium with which they were connected. The vessels thus reconstructed were found to be decidedly fewer in number than in the succeeding stages, and it is evident that no continuous plexus was present. It was noticeable that practically all of these vessels were connected with veins (fig. 32, plate 6).

In studying the region over the pelvis in chicks of 4 days 23 hours, in sections cut parallel to the surface, a very interesting condition was noted. This is the same region as that shown in figure 3, in a chick of 5 days 7½ hours. In the exact position of this delicate, continuous plexus of the older embryo we found a number of very fine vessels which did not contain any injection mass. A great many of these vessels were connected with blood-vessels. It might be inferred that such vessels are all new-forming blood-capillaries were it not for the difference in their appearance and location (for the most part beneath the superficial blood-vessels) and the fact that similar pictures were not found in the adjacent areas in which new formation of blood-vessels is also taking place. The character of these vessels is strikingly like that of those present in the earliest stage of lymphatic formation in the region of the posterior lymph-heart, recorded in figure 32, plate 6, and appears to show that this region over the pelvis also gives rise to new lymphatic vessels, and that here their origin occurs several hours later than the first formation in the tail region. Evidence obtained from the operations confirm this view and will be taken up later. The presence of numerous connections with blood-vessels appears to favor the view that the earliest lymphatics arise as outgrowths from the blood-vessels. Such communications with blood-vessels are not found in the later stages in which a continuous lymphatic plexus is present in this region.

And here we are forced to abandon the history of the first lymphatics of the chick, for the stage shown in plate 6, figure 32, is the youngest in which we were able to discover anything resembling a lymphatic. A chick of 4 days, measuring 9 mm., was sectioned parallel to the surface and this region was studied with the oil-immersion for a trace of possible lymphatic endothelium, but none was found. The intersegmental branches of the coccygeal veins have developed in the 4-day chick, but in the region of the later lymphatic plexus we could find no endothelium and no endothelial nucleus—nothing but a uniformly dense mesenchyme without the spaces present in later stages.

With regard to the much discussed question as to whether these first identifiable lymphatics arise from mesenchyme cells which differentiate into endothelial cells and send back processes to the veins, or whether they are outgrowths from the venous endothelium, we are unable to give a final and conclusive answer. The fact that, in the earliest stages in which the characteristic lymphatic endothelium can be identified, these first lymphatics are found to connect with veins in many places, while in later stages the number of such connections diminishes instead of increasing, appears to favor the view of outgrowth from the veins. But these studies do not exclude the possibility that for a very brief period mesenchyme cells may change into endothelial cells which form angioblasts and quickly acquire, first, a connection with the vein and then a lumen. It is to be hoped that some transparent region will eventually be found that will make it possible to watch the differentiation of these first lymphatics in the living and settle this question definitely.

Although the possibility that the first lymphatics may arise from mesenchyme *cells* is not excluded by these studies, their differentiation from mesenchyme *spaces* appears to be clearly untenable. These vessels appear at a time when the surrounding tissue is uniformly dense. The more open tissue directly adjoining the muscle, which in chicks of  $4\frac{1}{2}$  to 5 days contains spaces, is not invaded by the developing lymphatics. The stages showing the loose tissue containing the spaces formerly thought by some writers to be "pre-lymphatics," were found to be stages in which there is already a luxuriant plexus of lymphatics whose continuous endothelial lining and total independence of the spaces were demonstrated by all the methods used for these investigations. The isolated "pre-lymphatic" spaces, some of which were endothelial-lined and others apparently not, are quite evidently the result of incomplete reconstruction, inevitable when uninjected material and low powers of the microscope are used.

The first lymphatics occur in a dense region in which spaces are conspicuously absent, and the primitive plexus spreads out regardless of the character of the tissue which it invades. Everywhere this earliest plexus has the same form, consisting of delicate, string-like processes, minute thread-like connections, and larger, rounded, nodal points, the latter probably representing the result of beginning absorption. The plexus appears to be only secondarily influenced by the character of the tissue in which it is situated, and in chicks of 5 days and over those portions situated in a looser area, such as the lymph-heart region and the region anterior to the hind-limb, have larger vessels than those located in dense regions, such as the posterior tip of the pelvis and the axilla.

It is, of course, a great disappointment that we were unable, even by the use of such a painstaking method, to carry the history of the first lymphatics to a point at which the mode of origin of the lymphatic system could be established beyond a doubt; but we believe that the present studies have yielded valuable information with regard to the nature of the earliest lymphatics in stages in which the existence of such vessels had not been suspected. It has been possible to show that these vessels have definite morphological characteristics which distinguish them from other tissues. Also, the form of the beginning lymphatic plexus, with its many solid processes and its numerous mitoses, makes it appear that the method of growth of lymphatic capillaries, described by Clark (1909, 1912) for the transparent tails of amphibian larvæ, is also the method of growth of the primitive lymphatic system. In other words, the specificity of lymphatic endothelium has been traced back to a very early stage.

The history of the lymphatics of the chick in the region of the posterior lymph-heart has been traced back from the 8-day stage described by Budge (1882) and Sala (1900), in which a definite pulsating lymph-sac is present, to the stage of  $6\frac{1}{2}$  days in which the pulsations begin, although the heart is still in the form of a plexus; then to the stages of 5 to 6 days, in which a luxuriant blood-filled lymphatic plexus is present; to a still earlier stage in which a continuous plexus of finer vessels, without a continuous lumen, occupies this region; and finally, to a stage in which can be found a few very delicate vessels, most of which are connected with blood-vessels, and which have not yet formed a plexus.



The subsequent history of the superficial lymphatic system in chicks of  $5\frac{1}{2}$  to 9 days had previously been studied (E. L. Clark, 1915). By the method of injecting a very few granules of India ink directly into single lymphatic capillaries at various stages, it was possible to study the consecutive stages in the washing-out of the stagnant blood from the early superficial plexus with the establishment of lymph-flow, and also, simultaneously, the associated morphological changes. In this series of studies we found that the beginning lymph-flow in the various parts of the primitive superficial lymphatic system is accompanied by the differentiation of channels or lymph-ducts from the indifferent plexus, and that an increase in the flow is associated with an increase in the size and straightness of such ducts. The study also showed that in older chicks (8 days) lymph-sacs develop at a point where there are two conflicting pressures, and that such sacs are formed by the enlargement of a single lymph-channel or by the enlargement and coalescence of adjacent lymph-vessels. (Ranvier, 1896, 1897.)

Thus a continuous history has been obtained of the superficial lymphatic region in chicks of  $5\frac{1}{2}$  to 9 days, while in one typical region (that of the posterior lymph-heart) we have been able to complete the picture of the developing lymphatics from their first appearance as endothelium-lined vessels in chicks of 4 days and a few hours, up to the 9-day stage, when a heart with valves has developed. This study has shown that the important earlier investigations of the embryology of the lymphatic system, in which lymph-sacs connected with veins were thought to be the primary structures, the later studies in which the "veno-lymphatics" of these regions were pictured as forming an incomplete plexus, and the seemingly isolated vessels occurring elsewhere in the body, as well as the reconstruction of "spaces" in the path of developing lymphatics, were all mere fragments of the history of this system and subject to various interpretations. Again, the finding of a continuous plexus of independent lymph-capillaries connected with veins and filled with stagnant blood left a part of the history untold. The study with the oil-immersion lens of sections cut parallel to the surface and hence to the plane in which the lymphatics develop, and stained so as to bring out the contrasts between endothelial nuclei and the nuclei of mesenchyme cells, together with the injection of the blood-vessels, has enabled us to add another chapter to the history of the early lymphatics. This is a stage in which there is present a continuous plexus of vessels (some of them bulbous in shape, and others very narrow), which does not have a continuous lumen and which connects with the venous system in a number of places. Back of this we find a still earlier stage, the earliest so far described in birds or mammals, in which only a few vessels, most of them very delicate, are present in this region. Connections between these and the veins were found in practically every instance. These vessels do not form a continuous plexus. It is to be hoped that some future method will reveal the cells which are the direct ancestors of these earliest vessels with the characteristics of lymphatics, and thus complete the history.<sup>1</sup>

We now come to the second question which we attempted to solve with regard to the development of lymphatics—viz, the points of origin of the first lymphatics.

<sup>1</sup>The pictures of the earliest lymphatic vessels of the chick, obtained from these studies, are essentially similar to the earliest vessels of amphibians, as described by Fedorowicz (1913) and by Kampmeier (1915) and, like the results obtained by those authors, our observations favor the view that these earliest vessels arise as outgrowths from the endothelium of the veins.



II. OPERATIONS ON CHICK EMBRYOS.<sup>1</sup>

Earlier investigations (E. R. Clark, 1909, 1912) have shown that growth of lymphatic capillaries is by a process of sprouting from pre-existing endothelium, while the present study shows that the primitive lymphatic plexus, in the earliest stages of its development, increases rapidly in richness and by this process of growth undoubtedly invades near-by regions.

Many investigators have considered that the points of origin for the first lymphatics are limited to certain definite regions associated with veins. Thus Sabin (1901) originally described two points of origin for the lymphatics in pigs—the jugular lymph-sac and the iliac sacs (both paired). These were later increased by the addition of the retroperitoneal sac (single) discovered by F. T. Lewis (1906). All of the lymphatics of the body were thought to be derived from these points by the process of outgrowth. Other investigators differed from this view, considering that lymphatics have a diffuse origin. Huntington and McClure (1906, 1910) advocated the view that the origin of lymph-sacs is in certain definite regions associated with veins, while believing that other “peripheral” lymphatics arise diffusely from the mesenchyme. F. T. Lewis (1906) suggested that lymphatics arise diffusely from cut-off blood-vessels. Kampmeier (1915) describes the origin of lymphatic endothelium in *Bufo* as occurring diffusely by outgrowths from venous endothelium at many places.

We attacked the problem by the experimental method. By operating on chick embryos at a stage before any lymphatics had developed we removed various regions and then studied the modifications produced on the development of lymphatics. The questions we attempted to solve were the following:

1. Are the points of origin for the lymphatic system normally limited or diffuse?
2. If limited, where are these points of origin? Do they constitute only those regions in which the lymphatics are found to maintain connections with veins (the regions of the “primary lymph-sacs”)?
3. If the points of origin are normally limited, is it possible for lymphatics to develop *in situ* in other parts of the body when such points of origin have been removed? This would also answer the questions: Is the tissue (blood-vessel endothelium or mesenchyme) from which the first lymphatics differentiate *equipotential*, or is there some special quality of this tissue in one region which is not found in another?

In seeking the answers we began by removing the regions from which all of the superficial lymphatics have been thought to arise (Mierzejewski, 1909)—the region of the posterior lymph-heart in the tail, and the anterior region near the duct of Cuvier. These are the two regions where lymphatics connecting with veins had been demonstrated in chicks of 5 days and where these venous connections are maintained throughout embryonic life. The experiments performed were not of a kind to throw any light on the question as to the origin of lymphatics from mesenchyme or from blood-vessel endothelium, since both of these tissues were present before the development of lymphatics in all of the regions studied.

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<sup>1</sup>The operations on chick embryos were begun in the spring and summer of 1913, in the laboratory of Professor H. Hoyer, at the University of Cracow, Poland. The work for operation 1, and a part of the experiments recorded under operation 2, were completed at that time. It is a pleasure to have this opportunity to thank Professor Hoyer for his cordial hospitality, and for the extremely courteous, friendly, and stimulating interest which he displayed in our work during the very enjoyable months which we spent in his laboratory.

## METHOD OF OPERATION.

Description of the details of the operations must be left for the account of the individual experiments, since a number of modifications were adopted in each case. All of the operations were carried out under the binocular microscope inclosed in a warm chamber heated to incubator temperature. The operations were performed on chicks taken from the incubator to the warm box, where the shell was swabbed with cotton or cloth saturated in alcohol. As soon as the alcohol had evaporated a hole was pricked in the shell with a sterile needle. Into this opening the point of a pair of sharp forceps was inserted and the shell carefully picked off over an area about 6 mm. in diameter. A little warm sterile Ringer's solution was then dropped on the shell membrane, thus making the dissecting away of this membrane from the yolk much easier. Immediately after removing the shell the yolk sags away from the opening and it then becomes necessary to add, gradually, enough Ringer's solution to bring the embryo up to a level with the opening. If it is added too rapidly it may cause the embryo to rise out of the opening and result in a tear in the yolk membrane. If the region of operation is covered by amnion this membrane must be opened with forceps before proceeding. After the operation is finished the amnion can be "sutured" by pinching the edges together, whereupon it heals and continues to develop in a normal manner.<sup>1</sup>

The instruments used differed according to the type of operation performed; small iridectomy scissors were used when a portion such as the tail or wing bud was removed, while well-sharpened needles or a small knife with a triangular blade proved most useful in cases where it was necessary to dissect away somites or body-wall. In all cases sharp edges and fine points to the instruments are essential. Moderate aseptic precautions were used. The Ringer's solution was boiled and allowed to cool to a temperature of 37 to 39° C. before using. Needles and mica for the window were flamed, while more delicate instruments, such as the small forceps, knives, and scissors, were simply dipped in alcohol and allowed to dry.

After completing the operation more Ringer's solution was dropped into the opening. This helped to exclude air and to prevent sticking of the yolk-membrane to the ragged edges of the shell. A thin piece of mica was then placed over the opening and the edges sealed with a warmed mixture of beeswax (4 parts) and resin (6 parts). The egg was then returned to the incubator. The sticking of the yolk-membrane to the shell can be prevented if the egg is kept with the window side down, so that the embryo will float to the opposite side. It is also advisable to rotate the egg gently several times during the first few hours after the operation. The condition of the embryo (whether alive or dead), its stage of development, and hints as to the success of the operation can be observed from day to day by rotating

<sup>1</sup> A decided improvement in the technic of operating has been developed since this work was done. It was found that a considerable mortality is caused by the fact that, when the hole is made in the shell, the air is gradually forced out of the air-chamber until the chamber is obliterated. As a result a slight increase in the temperature of the egg after closing causes such an increase in pressure as to stop the heart-beat. If this is prevented by the simple expedient of immersing the egg during the operation in water at incubator temperature to a depth sufficient to cover the air-chamber, the air remains in the chamber, the chick does not sink away from the opening, and the mortality during the first 48 hours after operation is reduced nearly to zero. It is well to roll the egg slowly before operation, in order to make sure that the embryo is freely movable, and also to candle it in order to control the mobility and to determine the line of latitude on which to make the opening.

the egg so as to bring the window on top, and waiting for a few moments until the embryo moves around under the opening. The proper time at which to reopen the egg can also be determined in this way.

At the desired time, the mica was removed and the embryo again exposed. The presence of lymphatics was then tested by injections with India ink. In many of the experiments injection of the left side served as a control for the operated side. In some specimens the blood-vessels were subsequently injected with Berlin blue gelatin (5 per cent). The embryos were fixed in Carnoy's solution, dehydrated in three changes of absolute alcohol and cleared in benzol and oil of wintergreen. In cases where it was desired to section, the specimen was fixed in Bouin's fluid and stained by the same method described in Part I.

#### OPERATION 1. REMOVAL OF THE POSTERIOR LYMPH-HEART REGION.

The first operation consisted in the removal of the posterior lymph-heart region before the development of any posterior lymphatics. Since our other studies had made us familiar with the normal appearance of the lymphatic plexus which develops in this region, and since we had studied the invasion of the neighboring region (the posterior tip of the pelvis) by lymphatics connected with this plexus, this appeared to be the logical place for starting such an experiment. We therefore removed the tail in chicks ranging in age from 2 days 14 hours to 3 days, a stage at which 37 to 40 somites have differentiated. All of the tail region posterior to the thirty-third or thirty-fourth segment was snipped off with iridectomy scissors. The operation is comparatively simple, although a good deal of bleeding frequently follows the cutting. The total mortality amounted to 50 per cent, when precautions were developed for preventing the embryo from sticking to the shell. The eggs were returned to the incubator and development allowed to continue. They were reopened at intervals of 3 to 5 days after the operation, the lymphatics injected on both sides of the embryo, and their character and extent compared with those of normal chicks of a corresponding stage. Since healthy operated chicks were found, in most cases, to be delayed about 12 hours in their development, the injected embryos corresponded to chicks of 5 days 20 hours, up to 7 days 20 hours. These chicks developed with well-rounded stumps instead of tails, and the posterior lymph-hearts were absent.

In a normal chick of 5 days 20 hours lymphatics can be injected in the region of the thoraco-epigastric vein between the two limbs, where they form a plexus which connects through the axillary region with the deep jugular plexus, which in turn connects with the anterior and posterior cardinal veins in the region of the duct of Cuvier. Posteriorly, the lymph-heart plexus, with its venous connections and its superficial extensions over the posterior tip of the pelvis, can be injected (fig. 5). In operated chicks of this stage (6 days) the side plexus was readily injectible and entirely normal in appearance; but diligent search by means of many careful injection tests failed to reveal any posterior lymphatic plexus over the pelvis or stump. In embryos allowed to develop longer and examined when  $6\frac{1}{2}$  to 7 days old (or the stage corresponding to normal 6 to  $6\frac{1}{2}$  day chicks), a few ink granules

injected into one of the lymphatics of the anterior side plexus in the living chick moved anteriorly and disappeared in the axillary region, thus showing that lymph-flow had begun. Complete injection of these lymphatics showed that a plexus was present, normal in appearance for a chick of  $6\frac{1}{2}$  days, with a channel differentiated from the primitive plexus in the position of the path taken by the injected ink granules. More posteriorly an extensive plexus, continuous with the side plexus, was injected over the pelvis. At the posterior tip of the pelvis, however, where in a normal chick with a beating lymph-heart a rich plexus is present with one or more definite lymph-ducts differentiated, the operated chicks showed an indifferent, net-like plexus, composed of very fine capillaries with delicate, blind endings characteristic of the terminal border of a plexus.

In operated chicks which developed still farther, this most posterior portion of the superficial lymphatic plexus, while still primitive in character, was more extensive, and frequently the plexuses from the two sides were found to anastomose over the stump. No connections between the lymphatics and veins of this posterior region were injected in the operated specimens, no channels differentiated from the pelvic plexus, and in no case did a new lymph-heart differentiate.

In tailless chicks of the early stage of development, in which the early anterior plexus alone could be injected, stagnant blood was frequently found in the vessels of this region. No blood was ever found in the lymphatic plexus which appears over the pelvis in older embryos. By the time a continuous plexus from axilla to stump is present, the circulation has started in the side plexus and has washed the blood out of these lymphatics; while the posterior part of the plexus, having no connections with veins, does not receive any stagnant blood.

The results obtained from this operation are as follows:

When a point of origin for lymphatics (in this case the posterior lymph-heart region) is removed at a stage before any lymphatics have developed, the neighboring region (in this case the posterior tip of the pelvis), normally supplied from this point of origin, receives its lymphatic supply by ingrowth from another point of origin (more anterior lymphatics). This conclusion appears to follow from the delayed appearance of lymphatics over the posterior part of the pelvis and from the difference in their character shown by the delicate, blindly-ending tips. Although the region removed is one in which connections between lymphatics and veins are present normally from the earliest stage and maintained throughout embryonic life, the lymphatics which eventually develop over the stump in these tailless chicks do not show any connections with veins in injected specimens.

An interesting side result came from studying the conditions of lymph-flow in this series of operated chicks in comparison with the conditions present in normal

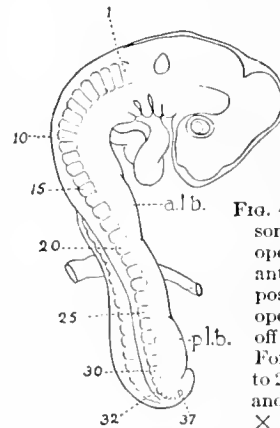


FIG. 4.—Chick of 3 days; 37 somites. The stage of operations 1 and 2. *a.l.b.*, anterior limb-bud; *p.l.b.*, posterior limb-bud. For operation 1 the tail was cut off at or near segment 32. For operation 2, somites 17 to 20 were dissected away and the wing-bud cut off.  $\times 12$ .

embryos. When the posterior lymph-heart fails to develop, the lymph-flow in the pelvic lymphatics does not start in chicks of 6 to 7 days and, coincidentally, definite lymph-channels do not appear in this region. On the other hand, in the anterior part of the superficial lymphatic plexus the circulation begins and the earliest ducts are formed in a normal manner.

OPERATION 2. REMOVAL OF ANTERIOR POINT OF ORIGIN FOR THE SUPERFICIAL LYMPHATICS.

As already stated, a plexus of lymphatics, situated beneath the shoulder and connecting with the veins in the region of the duct of Cuvier, was injected in chicks of 5 to 7 days. Connections from this plexus with the superficial side plexus through the axilla can always be injected in embryos of 5 days and older. This plexus, on account of its situation and relation to the venous system, has been thought to be homologous with the jugular lymph-sac of mammals (Sabin) and has been considered to be the point of origin for the lymphatics of the anterior part of the body, including those of the side region.

In starting experiments to remove this region of origin we first attempted to destroy the jugular (anterior cardinal) vein before the development of any lymphatics. This proved to be a rather difficult procedure and various methods were tried out and abandoned—the electric cautery and radium, because of the difficulty of localizing the burn; suturing of the vein with minute shreds of cloth, because of excessive injury to the delicate tissues, etc. We finally succeeded in removing the vein by the following method: Boiled and filtered aqueous Berlin blue was injected into the jugular vein in the head region. This material clumps on contact with the blood and sticks to the vessel wall, thereby plugging the vessel and at the same time rendering the wall visible. The injection was stopped at the proper moment so as to prevent the entrance of the blue granules into the heart. By this means the jugular vein, the duct of Cuvier, and a plexus of capillaries in the region of the posterior cardinal vein were injected and their circulation stopped. All of these injected vessels were then dissected away with sharp needles, and some of the tissue around them, as far anteriorly as the ear vesicle, was also removed.

This operation was performed on chicks of 42 to 48 hours. On examination 4 to 6 days later it was found that in every case a well-developed jugular vein was present on the operated side. In one case this was larger than the corresponding vein on the unoperated side. In other chicks it was somewhat smaller, but in all of them it was unquestionably present. It would seem that we were justified in concluding that conditions are present in the neck region which favor development of a large vein. This result, although of interest in connection with the problem of the development of blood-vessels, did not carry us any farther in the solution of the problems connected with the lymphatic system. In all of these specimens a normal plexus of lymphatics could be injected in the region of the thoraco-epigastric vein, which connected in a normal manner with a deep lymph-plexus, which, in turn, connected with the newly-regenerated jugular vein.

We next tried to isolate the side region, between the wing and leg, from the region beneath the shoulder, from which its lymphatics were supposed to be derived.

We first attempted to prevent the down-growth of lymphatics by inserting the tip of a porcupine quill into the angle formed by the duct of Cuvier and posterior cardinal veins in an embryo of 3 days, a stage at which this region is situated anterior to the shoulder. However, when such embryos were examined two or three days later it was found that this wedge had in no way interfered with the normal development, for the veins were present in their normal position beneath the shoulder, while the plug had remained in the neck region anterior to the shoulder. As might be expected, normal lymphatics were present over the side and connected with the deeper plexus in a normal manner.

Our next operation consisted in making a gap which would isolate the side region from the region associated with the veins beneath the shoulder. This was effected by removing the wing-bud and destroying a number of adjacent segments.



FIG. 5.—Normal chick of 5½ days, with superficial lymphatics injected with India ink. Compare with figure 6.  $\times 12$ .

FIG. 6.—Chick from operation 2, with superficial lymphatics injected. Embryo was operated on at 2 days 23 hours. Somites 17 to 20 were dissected away and the wing-bud removed. Three days later (when the chick was 5 days and 18 hours old) the egg was reopened and the lymphatics injected. Comparison with figure 10, which shows the superficial lymphatics of a normal embryo of the same stage of development, shows clearly that the side region of the operated chick has been effectually isolated from the deep anterior point of origin. In spite of this fact the lymphatics of the side region have developed normally.  $\times 12$ .

FIG. 7.—Chick of 2 days 18 hours which died soon after operation (operation 3). Six somites (22 to 27) were dissected away together with the body-wall opposite them. The tail-bud posterior to somite 32 was cut off.  $\times 12$ .

The wing-bud was snipped off with iridectomy scissors and the segments dissected away with fine needles and forceps. In the first operations of this series, in which only three of the somites were injured, the gap produced was not large enough, and the normal lymphatics of the side plexus, in chicks of 6 to 7 days, were found to connect along the dorsal side of the hole with the deep cervical lymphatics connecting with the veins. When four to seven segments, in addition to the wing-bud, were removed, the result was a large "chasm." In these embryos, allowed to develop two to three days after the operation, the jugular lymphatic plexus connected with the veins was present, but no vessels running posteriorly from it through

the axilla could be injected. In fact, the hole was of such a nature that there was no skin or subcutaneous tissue present for vessels to grow in. However, when the body-wall, between the gap and the posterior limb, was tested in such an embryo, *a normal lymphatic plexus was injected in every instance*. This extended anteriorly as far as the edge of the gap but could not be injected any farther. In such specimens (chicks of 5 to 5½ days) the posterior lymphatics were normal and were present in the lymph-heart region and near it; no lymphatics were injected over the pelvis. This condition is shown in figure 6 and can be compared with figure 5, showing the injected lymphatics of a normal chick of the same stage. Injection of lymphatics on the opposite (unoperated) side showed a similar condition, both in character and extent of the lymphatic plexus, except for the fact that axillary lymphatics, connecting the side lymphatics with the deep plexus, were present.

The fact that the side plexus in such operated specimens develops in the same region and at the same time as in normal embryos, and at a period before any growth from the posterior point of origin could have taken place, coupled with the fact of its completely normal appearance, speaks for the existence of another normal point of origin for the superficial lymphatics of the chick.

Observation of normal chicks at early stages (5 days and under) showed a stage in which a number of knobs of stagnant blood are present in that part of the lymphatic plexus of the side region just anterior to the leg. By injection these knobs were found to connect with a number of veins lying parallel to each other, which at this stage flow ventrally, encircling the abdomen. These veins are transitory; in chicks of 5½ days they disappear and the drainage for this region is all anterior, through the newly developed thoraco-epigastric vein—a stage at which a definite lymphatic plexus can be injected for the length of the side, with deep connections through the axilla with the jugular lymph-plexus. At this 5½-day stage and later no connections can be injected between these lymphatics of the side plexus and the neighboring blood-vessels.

It is also evident from these experiments that the regions in which lymphatic vessels retain their venous connections during all or part of embryonic life are not the only points of origin for the lymphatic system.

#### OPERATION 3. ISOLATION OF THE LEG AND PELVIS.

The foregoing experiments gave only a partial answer to the problems in which we were interested. We therefore tried to develop an operation for isolating completely a region supposed normally to be supplied by ingrowth from some point or region of origin. For this purpose we operated on chicks toward the second half of the third day of incubation (embryos averaging 2 days and 17 hours), removed the tail region as in operation 1, and also dissected out the five or six somites posterior to the omphalo-mesenteric vein and the adjacent body-wall on the right side. By this means we succeeded in obtaining embryos without tails (with the posterior point of origin removed) in which a gap had been made on one side, isolating the leg and pelvis from the anterior lymphatics, and in which the probable point or region of origin anterior to the leg had been removed. More than a dozen chicks survived this double operation.



On examining an embryo of this kind which had been allowed to develop until it was 7 days old we found a gap in the body-wall anterior to the leg which extended dorsally as far as the dense tissue around the spinal cord (where lymphatics are never found at this stage), and which exposed the abdominal viscera. The amnion was often attached along the borders of the gap.

Injection of the left side of the embryo (the unoperated side except for the removal of the tail) showed a continuous lymphatic plexus which extended from the deep jugular plexus connected with veins, through the axilla, down the side, over

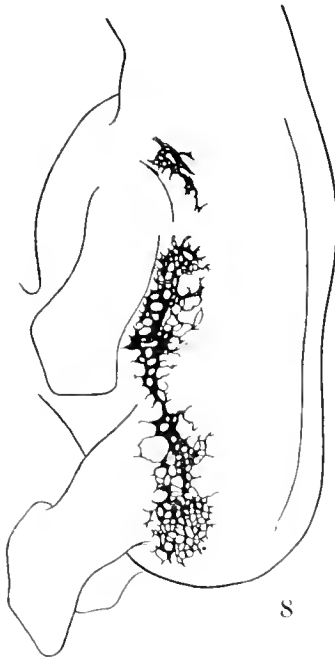
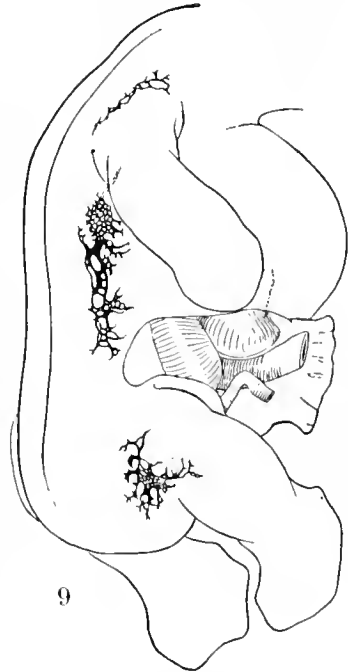


FIG. 8.—Left side of a chick of 6 days. This side is normal except for the absence of the tail. Continuous superficial lymphatic plexus injected with India ink. This serves as a control for the right side of the same embryo shown in figure 9, which is an example of operation 3.  $\times 12$ .

FIG. 9.—Right side of same embryo shown in figure 8. Chick of 6 days. Example of operation 3. At 2 days and 12 hours the six segments anterior to the posterior limb-bud of this embryo were dissected away, together with the body wall opposite. The tail was then cut off. Lymphatics injected with India ink. The vessels anterior to the gap are normal in appearance and extent. Over the posterior part of the pelvis a lymphatic plexus, normal in appearance but less extensive than usual, was injected. No lymphatics were injected on the anterior portion of the pelvis or over the stump. Compare with the lymphatics of the opposite side shown in figure 8.  $\times 12$ .



the pelvis and to the posterior stump, where the vessels were fine and delicate. Lymphatics were also present in the region dorsal to the shoulder connecting, anterior thereto, with the deep jugular plexus, but not yet posteriorly with the side lymphatics. In other words, this left side possessed a lymphatic plexus of the type described in operation 1, normal everywhere except for the absence posteriorly of the beating lymph-heart and the consequent primitive character of the vessels over the posterior tip of the pelvis (fig. 8).

On the right side our injections showed anteriorly the presence of normal lymphatics in the deep jugular region, in the region dorsal to the shoulder, in the axilla, and also in that portion of the side region anterior to the gap. Here, at the edge of the gap, the lymphatic injection ended in a number of delicate points. Repeated injections over the anterior part of the pelvis and leg in operated chicks of this stage failed to show any lymphatics corresponding to those present in this region in normal embryos or to the plexus already injected on the opposite side. But by plunging the cannula into the tissue more posteriorly, over the proximal part of the pelvis, a plexus of lymphatic capillaries of normal appearance, richness, and location was injected with ease (fig. 9).



This operation was repeated a number of times, and by allowing the chicks to incubate for varying periods, we obtained an interesting series of embryos.

In an operated embryo of 5 days and 15 hours, corresponding to a normal chick of about  $5\frac{1}{2}$  days, injection revealed a normal anterior plexus on the left side extending deep into the axilla. As in normal chicks, the lymphatics dorsal to the shoulder had not yet developed. Posteriorly, the lymph-heart region was absent and no lymphatics could be injected over the pelvis. In other words, the condition of lymphatics was identical with that found in embryos after operation 1.

On the right side, where the double operation had been performed, the lymphatic plexus anterior to the gap was present and normal in appearance. It extended from the deep jugular plexus connecting with the veins, posteriorly through the

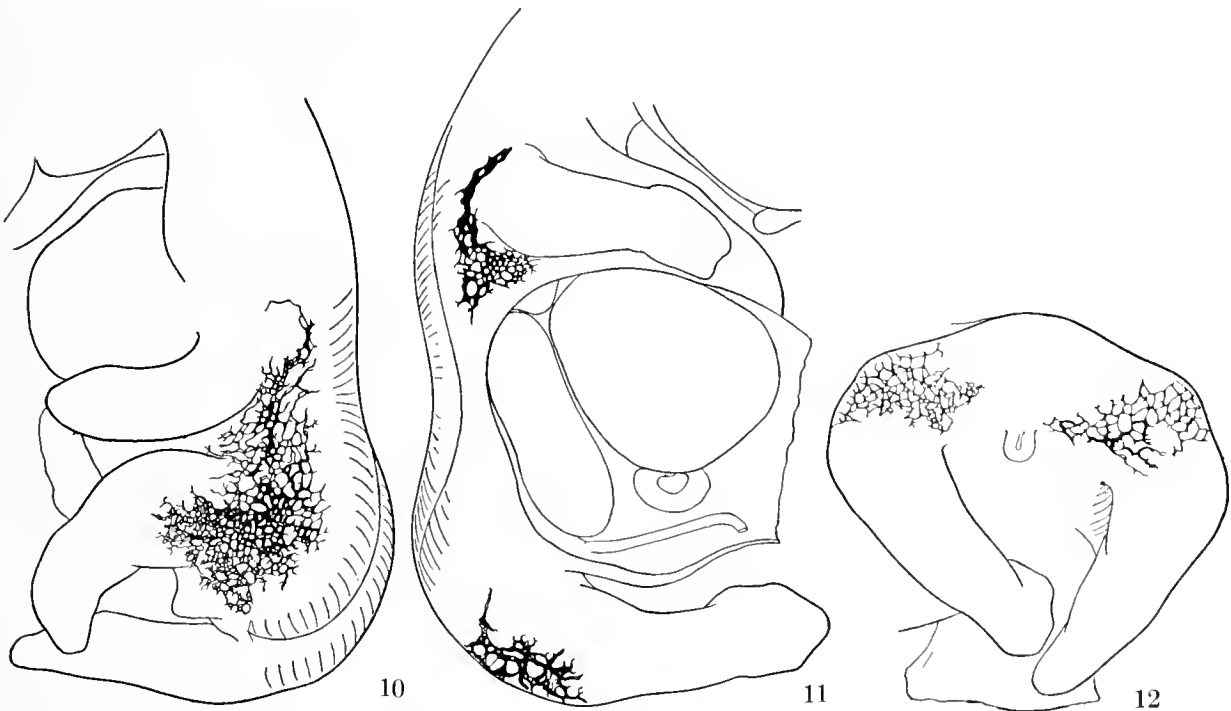


FIG. 10.—Left side of an embryo of 6 days and 15 hours. Normal except for the absence of the tail. Superficial lymphatic plexus injected. Control for right (operated) side shown in figures 11 and 12.  $\times 12$ .

FIG. 11.—Right (operated) side of a chick of 6 days and 15 hours. Same embryo as in figure 9. Large gap present in body-wall extending to mid-line of the back, leaving spinal cord exposed. Embryo twisted toward left side. Lymphatic plexus injected dorsal to shoulder and in axillary region normal. Posteriorly, lymphatic plexus injected on posterior half of pelvis and around the stump (fig. 12). Normal in character. No lymphatics injected over anterior part of pelvis.  $\times 12$ .

FIG. 12.—Posterior view of the same embryo shown in figures 10 and 11, showing the lymphatic plexus extending around the tail-stump from both sides.  $\times 12$ .

axilla, and ended just anterior to the gap in fine delicate points, smaller than the vessels usually found in this region. Posterior to the gap—*i. e.*, over the leg and pelvis as on the opposite side—no lymphatics could be injected.

The next stage found in this experiment is that which has already been described—the chick pictured in figures 8 and 9.

In the next stage (an embryo of 6 days and 15 hours) the left side showed a luxuriant lymphatic plexus from the axilla, over the side, the pelvis and almost

around the stump (fig. 10). The anterior portion of this plexus was entirely normal, as was also the posterior portion, except for the absence of large channels over the posterior tip of the pelvis and the presence in their place of a more primitive plexus with fine delicate endings.

On the right side there was a very large gap which bordered dorsally on the spinal column and left the entire Wolffian body and most of the liver exposed. The lymphatic injection showed a normal plexus dorsal to the shoulder, connected anteriorly, as on the left side, with the deep cervical plexus, and posteriorly with the side lymphatics. This plexus was not extensive, owing to the large size of the gap. It connected anteriorly through the axilla with the deep jugular plexus, and posteriorly ended blindly in numerous fine points at the edge of the gap. On the anterior border of the pelvis (in the region just posterior to the gap), as in the younger chicks just described, no lymphatics were injected. Farther posteriorly on the pelvis, however, a large plexus of lymphatic capillaries was injected. This was normal in appearance and more extensive than that shown in fig. 9. It extended over the tip of the pelvis, ending, like the plexus of the opposite side, near the middle of the stump in numerous finely-pointed, blind tips (figs. 11 and 12).

A still older stage is shown in figure 13, a chick of 7 days. The lymphatics of the left side resembled those shown in figure 10, except for the presence of some larger ducts in the side region between the limbs. On the right side the lymphatics of the shoulder region, and of the side region anterior to the gap, were normal for chicks of this stage. Over the pelvis, from the region just posterior to the gap and extending well around the stump, was a luxuriant plexus of lymphatics resembling those of the opposite side in character and extent. No connections between this posterior lymphatic plexus and the veins could be found in any specimen after this type of operation. It should be emphasized, however, that no microscopic studies were made on the operated chicks at the younger stages in which probable venous connections were found in this region in normal chicks.

This series of specimens shows that there is apparently still another point of origin for the superficial lymphatics of chick embryos—*i. e.*, a region over the posterior part of the pelvis. The lymphatics which develop here make their appearance somewhat later than those in the jugular region, the side region anterior to the leg, or those which differentiate in the posterior lymph-heart region of the tail.



FIG. 13.—Right side of an operated embryo of 6 days 18 hours, showing a later stage of development of the superficial lymphatics after operation 3. Operative procedure same as that described under figure 12. Injected lymphatics in the suprascapular and axillary regions normal in appearance. The lymphatics posterior to the gap, in this specimen, cover the whole pelvis and stump just as on the left (unoperated) side.  $\times 12$ .

This is evident from injections of normal embryos, in which the delicate plexus along the side, the deep plexus near the duct of Cuvier, and the lymph-heart plexus, all can be demonstrated before any lymphatics over the pelvis can be injected.

Our studies of oil-immersion reconstructions also gave evidence of the later differentiation of these lymphatics. For example, at the stage shown in plate 5 the lymph-heart plexus and a few extensions (probably outgrowths from it) are shown as a continuous plexus. More anteriorly, in the region where the pelvic plexus is found in older embryos, we were able to reconstruct a number of fine vessels, some of them connected with blood-vessels but differing in appearance from blood-vessel sprouts, and others apparently isolated. About eight hours later, as shown in figure 3, a continuous lymphatic plexus is present throughout the region over the posterior half of the pelvis, which connects with the lymphatic plexus in the tail. This continues to grow rapidly in extent and richness, as evidenced by the many solid processes at the edge of the plexus and the numerous mitoses in the endothelial cells. The connections with blood-vessels, found in these sections in the earliest stages, evidently are soon lost, since none can be injected in the later stages in which a continuous lumen is present.

These observations support the conclusion drawn from the results of the operation just referred to, that there is a separate point of origin for the superficial lymphatics over the posterior part of the pelvis.

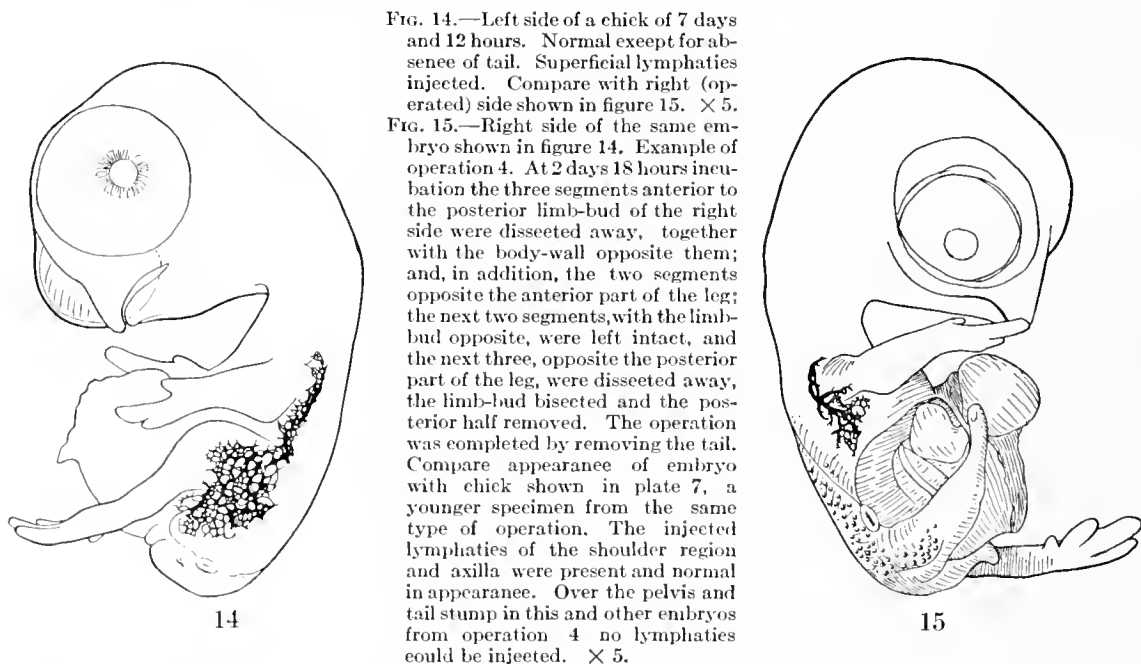
#### OPERATION 4. ISOLATION OF THE ANTERIOR PART OF THE LEG AND PELVIS.

Although operations 2 and 3 had yielded interesting results in regard to the points of origin for the superficial lymphatics, a conclusive answer had not yet been given to the other problem, *i. e.*, whether lymphatics can develop in a region which has been completely isolated from its normal source of lymphatic supply. The evidence obtained from injections of normal embryos, and from experiment 3, apparently showed that the anterior portion of the leg and pelvis receives its lymphatic supply from the anterior lymphatic plexus of the body-wall in the case of normal chicks, or by invasion from the posterior part of the pelvis when this anterior source of supply has been removed. We therefore attempted a still more radical operation—the effective isolation of this region. This entailed the removal of several segments (three to seven) and the body-wall adjacent to them in the region just anterior to the right hip. The procedure was a repetition of that used in operation 3. Then, the anterior part of the posterior limb-bud, together with the somites opposite, being left intact, the remainder of the leg and the adjacent somites on that side were dissected away. The operation was completed, as in operation 3 by snipping off the tail, the aim being to completely isolate the anterior part of the leg and pelvis of the right side from the area of lymphatic origin of that side, and also from that over the posterior part of the pelvis. The left side of the embryo was allowed to develop in a normal manner except for the absence of the tail, including the lymph-heart region.

In the first two experiments of this kind three somites opposite the anterior half of the right leg were allowed to remain intact. These embryos developed

without tails, with a large gap in the body-wall on the right side anterior to the leg, and with the right leg smaller than usual and possessing two toes. On the left side a continuous plexus of superficial lymphatics was injected over the side, pelvis, and stump. On the right side the plexus anterior to the gap in the axillary region was normal in appearance, and over the posterior part of the pelvis and stump a normal lymphatic plexus was injected with ease. In other words, the result was identical with that obtained in experiment 3. The operation was repeated in the same manner except that in this case only two somites were left untouched in the region opposite the anterior portion of the right leg, and the leg was bisected, the posterior half and the three remaining segments opposite it being removed.

In this operation 6 embryos were opened at varying incubation ages. Plate 7 and text-figures 14 and 15 illustrate their appearance at 7 and  $7\frac{1}{2}$  days respectively.



The gap anterior to the leg and the rounded stump characteristic of operation 3 are noticeable, and in addition the right leg may be seen to be deformed and to possess only one toe.

Four of the embryos were opened at  $6\frac{1}{2}$  to  $7\frac{1}{2}$  days (the stage illustrated in plate 7, figure 33) and the lymphatics injected. The left side was tested first and the injection showed the presence of the continuous plexus described in operations 1 and 3 (left side). On the right side normal lymphatics were injected in the supra-scapular region, in the region beneath the shoulder, in the axilla, and as far posterior as the gap in the body-wall. However, when the region over the pelvis was tested, repeated careful injection *failed to show any sign of a lymphatic plexus*. The injected ink, instead of remaining in blebs, as is usual in the case of extravasations in the subcutaneous tissues, spread out rapidly through the tissue in finger-like projections which finally ran together and made a continuous black sheet. That

the failure to inject the lymphatics in the right side was not due wholly to the condition of the tissue is evidenced by the fact that in many of these embryos the tissue over the pelvis of the left side was more edematous than that of the right side, and yet lymphatics were always injected on the left side at the first insertion of the cannula.

The results of the injection tests in operation 4 made it appear as if the answer to this part of our problem had at last been found. One of these embryos (shown in plate 7, fig. 33) was selected for sectioning. As may be seen, the gap in the body-wall anterior to the leg was sufficiently large to isolate the leg and pelvis from its anterior source of supply, while the rounded stump and deformed leg, with only one digit, exactly resembled the condition present in the four other embryos just described. In this specimen we refrained from testing for lymphatics the region over the pelvis of the right side, since we feared that the extravasations thus produced might obscure the cytological picture. On the left side we injected a small amount of Berlin blue, enough to demonstrate the presence of a normal lymphatic plexus. The blood-vessels were then injected completely, the embryo was fixed in Bouin's fluid, dehydrated, cleared, and sectioned according to the method described for the studies in Part I.

In studying the sections of the pelvic region we found a number of irregular vessels which had not received the injection mass. In reconstruction it was found that these vessels were located, for the most part, in the layer beneath the superficial blood-capillaries, some of them coming to the surface in the interstices between blood-vessels. From their location and general appearance there appeared to be no doubt that these vessels were indeed lymphatics. They differed considerably in character from normal lymphatics of this stage and were not so large or nearly so numerous as the lymphatics of the opposite side. Although these vessels formed a plexus in places, this resembled the more primitive type formed by the earliest lymphatics described in Part I, except for the greater size of some of the component vessels. Many of the connections were very narrow and others were thread-like and solid. The absence of any extensive lymphatic plexus over the pelvis after operation 4, and the lack of any continuous lumen in the scanty plexus which was present, undoubtedly accounted for our failure to inject lymphatics in other chicks of this series. It is significant that these are the same factors which prevented injections of the earliest lymphatics described in Part I.

This straggling lymphatic plexus, peculiarly primitive in many respects and much less luxuriant than usual, was for the most part independent of the surrounding blood-vessels. However, certain of these irregular vessels possessed an undoubted connection with an injected blood-vessel. They were in no wise distinguishable from the other vessels of the plexus and differed markedly from the straight, regular, and narrow blood-vessel sprouts. Moreover, no such "sprouts" were found on the left side of this embryo, where, as has been indicated, a normal and extensive lymphatic plexus interlaced with the blood-capillaries without communicating with them. From the earlier studies, reported in this paper, of the characteristics of the earliest lymphatics and their relation to blood-vessels, there

seemed to be some evidence in this specimen that these lymphatic vessels (clearly recently formed structures) were in process of differentiation from blood-vessels; but, as was the case in the study of the differentiation of the lymphatics of the lymph-heart region, we can state only that such a theory can not be settled definitely from a study of fixed material.

The convincing points obtained from the study of sections of an embryo operated on in this manner are:

(1) That undoubted lymphatics are present in a region which had been effectively isolated from all known points of origin for its lymphatics.

(2) That these lymphatics are abnormal in appearance for this stage, and, in comparison with those of the unoperated side, resemble primitive lymphatics to such a degree that it would seem highly probable that they were undergoing the early development described for the first lymphatics of the posterior lymph-heart region. The greater size of some of the vessels of this plexus is probably attributable to the greater looseness of the surrounding tissue and to the consequently greater expansion of these newly-formed vessels, for it will be remembered that the earliest lymphatics normally differentiate in a comparatively dense tissue.

Thus, in operation 4 it was shown that lymphatics will develop *in situ* in a region that has been completely isolated from the points of origin from which, presumably, it would normally receive its lymphatic supply. These vessels are not normal for this stage of development and do not resemble those of the unoperated side. From the fact that, unlike the normally appearing plexus found in embryos after operation 3, no plexus of vessels was injected, and also from the appearance of these lymphatics in sections, they appear to be primitive vessels which develop *in situ* in a manner comparable with that described for the earliest lymphatics of the posterior lymph-heart region. The development of such lymphatics is evidently delayed in this case, since only these primitive vessels are present at a stage in which the opposite side possesses an abundant lymphatic plexus.

#### SUMMARY OF RESULTS OBTAINED FROM OPERATIONS ON CHICK EMBRYOS.

The experiments just recorded have given the following answers to questions of the points of origin for the superficial lymphatics of chick embryos:

1. Regions of origin for the superficial lymphatics of chicks are *not* limited to those areas in which venous connections are retained during embryonic life—*i. e.*, to the regions of the so-called primary lymph-sacs which, in the chick, are beneath the shoulder and are associated with the veins near the duct of Cuvier, and the tail region associated with the first five coccygeal veins.

2. There are at least four such regions of origin for the superficial lymphatics of chick embryos: the two mentioned above, where the venous connections are retained, and two others, one in the side region, probably that portion just anterior to the leg, and another on the posterior part of the pelvis. In the latter two no venous connections can be demonstrated after the early lymphatic plexus has become continuous over the surface of the body (operations 2 and 3).

3. Such points of origin are not restricted in size; in fact the areas in which differentiation of superficial lymphatics takes place appear to be fully as large as the regions which are supplied by ingrowth.

4. When a region of origin is removed by operation, at a stage before lymphatics have started to develop, the adjacent region usually supplied by extension of lymphatics from this point of origin receives its lymphatic supply by ingrowth from another source (operation 1).

5. When the point of origin removed is one of those in which venous connections are normally retained (such as the posterior lymph-heart region) no new permanent connections with veins develop. In this case a beating lymph-heart never develops elsewhere to replace the one whose anlage has been removed (operation 1).

6. When a region is effectively isolated from all its usual sources of origin, lymphatics will eventually develop *in situ* (operation 4).

In a word, the results of the operations justify the general conclusion that, while the formation of permanent connections between the lymphatic system and the veins is apparently restricted to certain definite regions, the differentiation of lymphatic endothelium is not so restricted, but occurs at many places in the embryo.

#### GENERAL CONCLUSIONS.

The modern work initiated by the studies of Ranvier, Sabin, and MacCallum, has shown that the lymphatic system is composed of definite vessels which are everywhere separated from the spaces and cells of the connective tissue by an endothelial membrane. The discovery that this membrane is present throughout the lymphatic system has made necessary a revision of the older terms for certain fluids of the body, all of which had been known as *lymph*, and a new division into (1) plasma, (2) tissue fluid, and (3) lymph, designating respectively the fluid inside the blood vessels, the fluid of the tissue spaces (including the cerebro-spinal fluid and the fluid of the serous cavities) and the fluid inside the lymphatics (Sabin, 1916). The basis for this new conception of the lymphatic system, with the emphasis which it throws upon the importance of endothelium, rests chiefly on the embryological studies begun by Sabin and carried on by numerous other investigators. These morphological studies have shown that lymph-vessels invade the different regions and organs of the embryo by a process of ingrowth, and observations on the transparent tails of living tadpoles have established the fact that lymphatic capillaries remain completely independent of the surrounding cells and tissues and grow by a process of sprouting from preëxisting lymphatic endothelium. It has also been shown, in studies of chick embryos, that the primitive form of the lymphatic system is an indifferent, net-like plexus, out of which ducts and sacs develop secondarily in response to pressure conditions inside and outside the vessels. A beginning has been made toward finding out some of the reactive powers of lymphatic endothelium: it is known to be phagocytic and to be capable of responding to certain substances (by growing toward them) which do not stimulate a similar response on the part of the blood-capillaries.

The point in the development of the lymphatic system at which this lymphatic endothelium becomes the specific tissue present in the stage represented by the lymphatics of the tadpole's tail, and the manner in which this tissue originates, are questions which have been investigated extensively but never completely settled.

In these observations it has been found that in chick embryos the lymphatic capillaries may be identified as definite endothelium-lined vessels in embryos of  $4\frac{1}{2}$  days, a much earlier stage than any previously described. These early vessels possess endothelial nuclei with morphological characteristics which distinguish them from the nuclei of the mesenchyme cells. The earliest lymphatics are tubes and strands of endothelium, most of them connected with blood-vessels. They have a marked tendency to plexus formation and the resulting plexus in its primitive form is made up of vessels of irregular shape and size, bulbous portions alternating with fine sprouts and solid connections of extreme delicacy. The continuous endothelium precedes the formation of a continuous lumen in the primitive lymphatic plexus. Mitotic figures are numerous in these lymphatics, thus showing that the method of growth by sprouting is acquired very early.

The present studies exclude the possibility that lymphatics are formed from spaces in the connective tissue. The numerous connections between the earliest lymphatics and blood-vessels, the majority of which are lost within the first 30 hours of lymphatic development, make it appear probable that the first lymphatic vessels have arisen from the endothelium of blood-vessels. However, the possibility that the differentiation of lymphatic endothelium may occur in the manner recently described for the first origin of blood-vessel endothelium (Stockard, Sabin) has not been excluded by the methods of study used in this investigation.

Our studies have also shown that the points of origin for lymphatics are by no means confined to those regions in which lymph-sacs connected with veins are present in older embryos. The operations on chick embryos have demonstrated at least four regions of origin for the superficial lymphatics. These experiments further show that the character of the blood-vessel endothelium (or mesenchyme) is *not* specialized in certain limited regions as regards its ability to give rise to lymphatic endothelium; for when a region, normally supplied by ingrowth, is effectually isolated from its sources of supply, lymphatics will eventually develop *in situ*, but whether from blood-vessel endothelium or mesenchyme cells was not determined.



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## DESCRIPTION OF PLATES.

### PLATE 1.

- FIG. 16. A living chick of 5 days 16 hours. Measurement 16.5 mm. (fresh specimen).  $\times 5$ . The square indicates the region shown in figure 17.
- FIG. 17. Posterior part of pelvis and tail region of same embryo, showing the two sets of superficial vessels visible in living specimen. Blood-capillaries orange-red in color, lymphatics deep red. The arrow indicates direction of blood-flow in the blood-capillaries. The blood in the lymphatics is stagnant.  $\times 24$ .
- FIG. 18. Chick of 5 days 12 hours.  $\times 3$ . The portion posterior to the dotted line is the region illustrated in figure 19.
- FIG. 19. Tail and posterior pelvic region of the same embryo. The blood-vessels were injected through one of the vitelline veins. The lymphatic plexus remained filled with stagnant blood. The drawing was made from the fresh specimen immediately after injection. The deeper lymphatics of the posterior lymph-heart plexus are shown in paler red, the more superficial capillaries in brighter red.  $\times 18$ .

### PLATE 2.

- FIG. 20. Chick of 5 days 22 hours. The lymphatic plexus in the posterior lymph-heart region and over the posterior tip of the pelvis, is injected with India ink.  $\times 24$ . The square indicates the region shown in figure 21.
- FIG. 21. Tail and posterior pelvic region of the same embryo. The lymphatic plexus in the posterior lymph-heart region, and the more superficial plexus connected with it, were injected directly with India ink without disturbing the neighboring blood-vessels. A—superficial lymphatic plexus; B and C—lymph-heart plexus; D—points at which cannula was inserted to make injections.  $\times 36$ .
- FIG. 22. Posterior half (left side) of a chick 5 days 15 hours old. Measurement before fixation 16 mm.; blood-filled lymphatic plexus present in the tail region and over the pelvis and hip. The embryo was bled and the drawing of the blood-filled lymphatic plexus made from the fresh specimen.  $\times 36$ .

### PLATE 3.

- FIG. 23. Chick of 5 days 18 hours.  $\times 2.5$ . The portion posterior to the lines is the region shown in figure 24.
- FIG. 24. Tail and posterior pelvic region of the same embryo. Double injection. Blood-vessels completely injected with India ink (black in the drawing). Lymphatics injected with silver nitrate 0.5 per cent (white in the drawing). The lines A and B indicate the plane of the section shown in figure 25.
- FIG. 25. Thick cross-section through the tail region of the same specimen; i.e.a.—intersegmental coccygeal artery; i.e.v.—intersegmental coccygeal vein. The superficial blood-vessels are dark in the drawing and the lymphatics white as in figure 24.  $\times 36$ .

### PLATE 4.

- FIG. 26. Microscopic drawing (oil immersion) of a portion of the lymph-heart region of a chick of 4 days 23 hours, measuring  $12\frac{1}{4}$  mm. before fixation. The blood-vessels were completely injected with India ink. The specimen was sectioned parallel to the surface and the sections stained with Ehrlich's hematoxylin, counter-stained with eosin, orange G. and aurantia. The figure shows a portion of a lymphatic capillary and of a blood-capillary, containing ink granules, and the adjacent mesenchyme cells. lym.—lymphatic; l.n.—lymphatic nucleus; b.v.n.—blood-vessel nucleus; m.n.—mesenchyme cell nucleus.  $\times 1,200$ .
- FIG. 27. Higher power drawing of the nucleus of a lymphatic endothelial cell (l. n.), and of the nucleus of a mesenchyme cell (m. n.).  $\times 1800$ .
- FIG. 28. Microscopic drawing of a chick of 5 days  $\frac{1}{2}$  hour, showing an early lymph-vessel of the posterior lymph-heart region and its connections with an injected blood-vessel; lym.—lymphatic; b.v.—blood-vessel; con.—connections between lymphatic and blood-vessel; l.n.—lymphatic nucleus; r.b.c.—red blood-cells inside of the lymphatic vessel.  $\times 800$ . (Magnification of original drawing 1 to 1200.)
- FIG. 29. Microscopic drawing of a chick of 4 days 23 hours, showing an early lymph-vessel of the lymph-heart plexus with an endothelial nucleus undergoing mitotic division.  $\times 800$ . (Magnification of original drawing 1 to 1200.)

### PLATE 5.

- FIG. 30. Oil immersion reconstruction of the vessels of the lymph-heart region in a chick of 4 days 23 hours; measurement  $12\frac{1}{4}$  mm. before fixation. The early lymphatic plexus is shown in blue. The injected blood-vessels are shown in lines. X, connections between lymphatics and blood-vessels.  $\times 320$ . (Magnification of original drawing 1 to 800.)

### PLATE 6.

- FIG. 31. Oil immersion reconstruction of the vessels of the lymph-heart region in a chick of 4 days  $9\frac{1}{2}$  hours; measurement 11.75 mm. before fixation. Probable lymphatics in blue. Injected blood-vessels in lines. X, connections between lymphatics and blood-vessels.  $\times 320$ . (Magnification of original drawing 1 to 800.)
- FIG. 32. Oil immersion reconstruction of the earliest lymph-vessels in the region of the posterior lymph-heart of a chick of 4 days  $7\frac{1}{2}$  hours. Measurement before fixation 9 mm. The probable lymph-vessels are blue. Injected blood-vessels indicated in lines.  $\times 320$ . (Magnification of original drawing 1 to 800.)

### PLATE 7.

- FIG. 33. Operated chick of 7 days. At 2 days 14 hours incubation five segments anterior to the posterior limb-bud of the right side were dissected away. The next two segments and the anterior half of the limb were left intact, and the next succeeding three segments, opposite the posterior part of the leg, were removed, together with the posterior half of the leg. The tail was then removed. This is an example of the type of embryo obtained from operation 4. X indicates an exposed portion of the spinal cord located at about the midline of the back.  $\times 20$ .

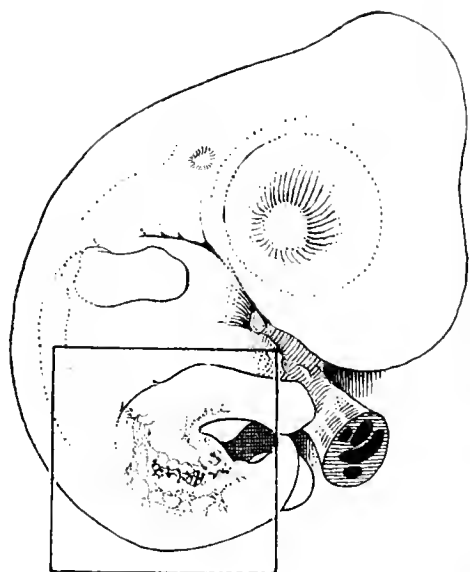


Fig. 16



Fig. 17

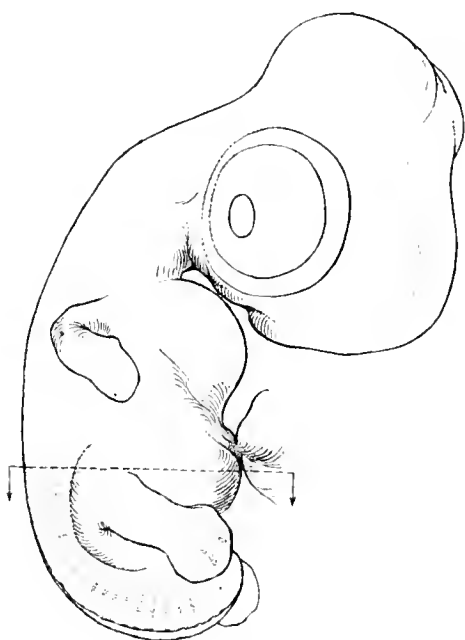


Fig. 18

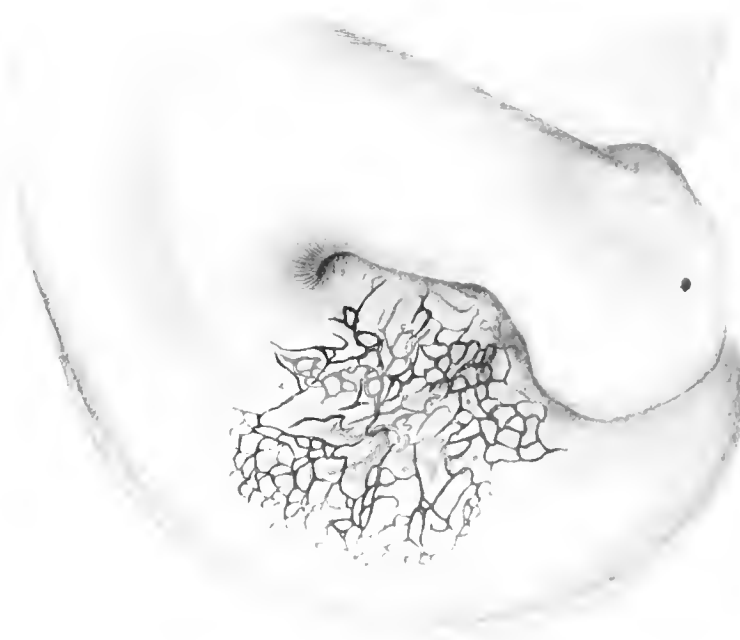


Fig. 19



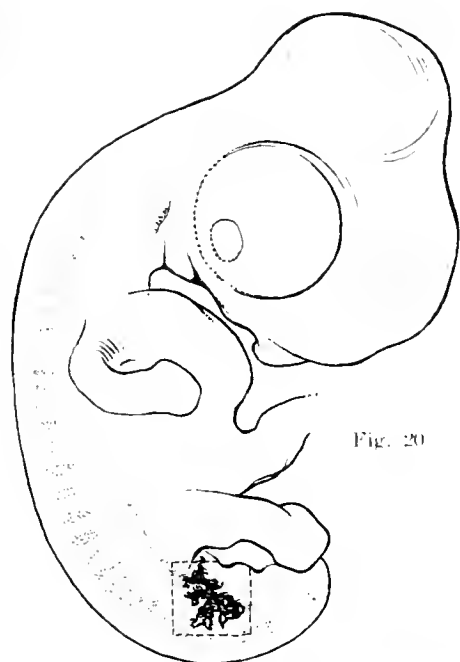


Fig. 20

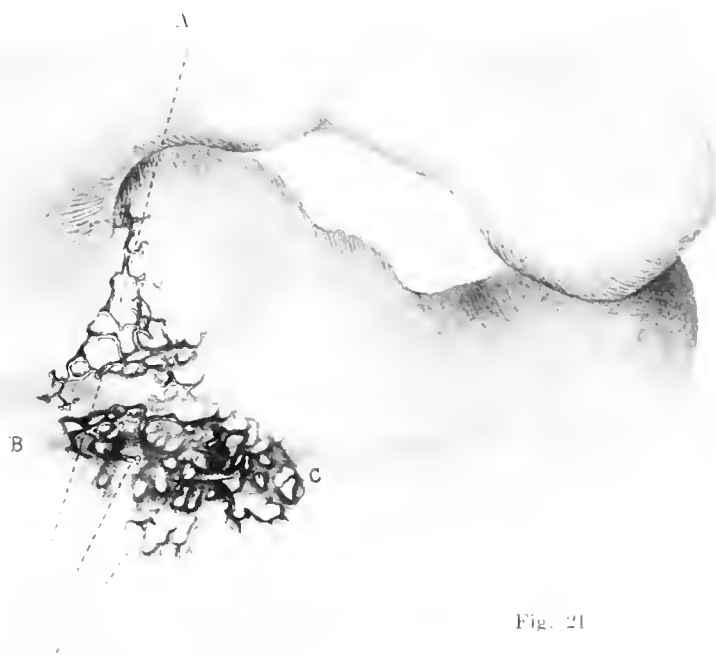


Fig. 21

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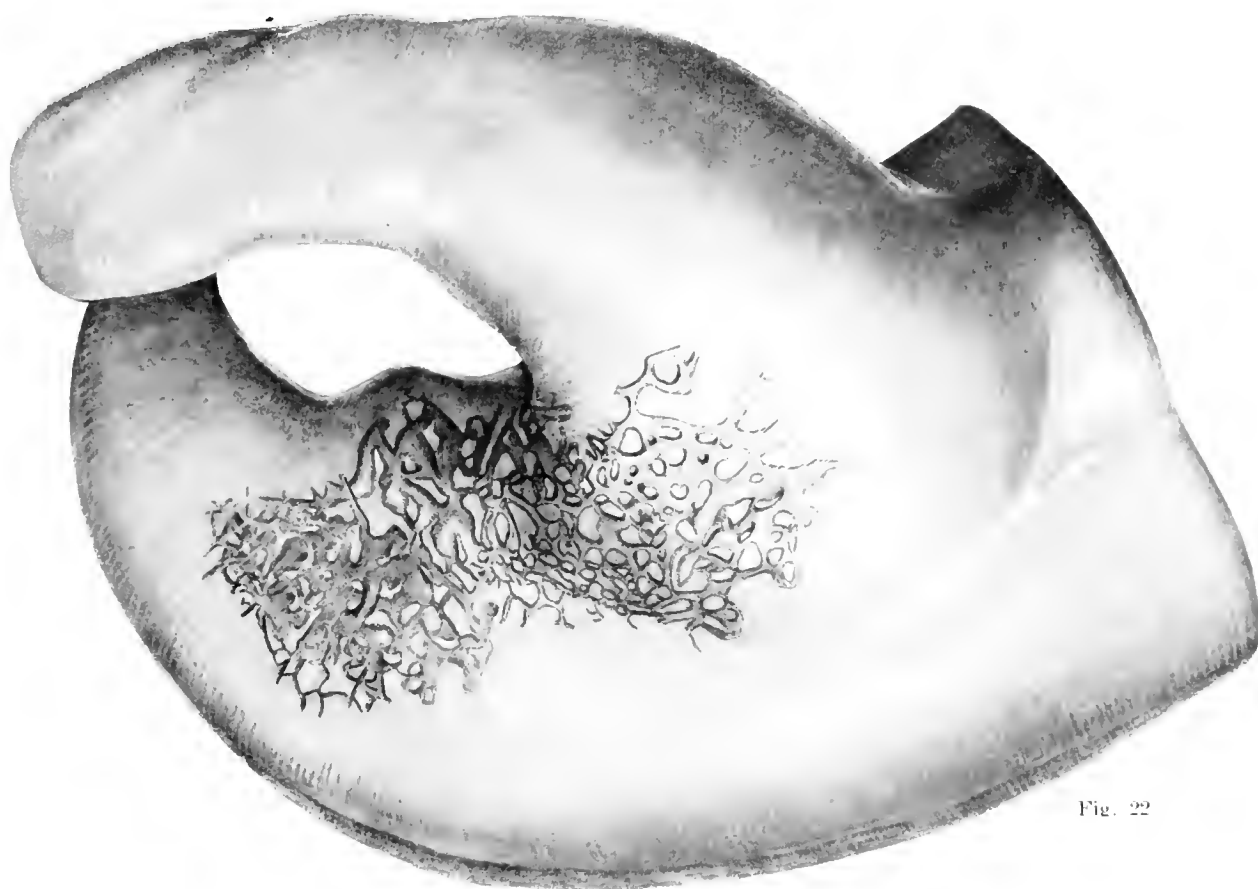


Fig. 22



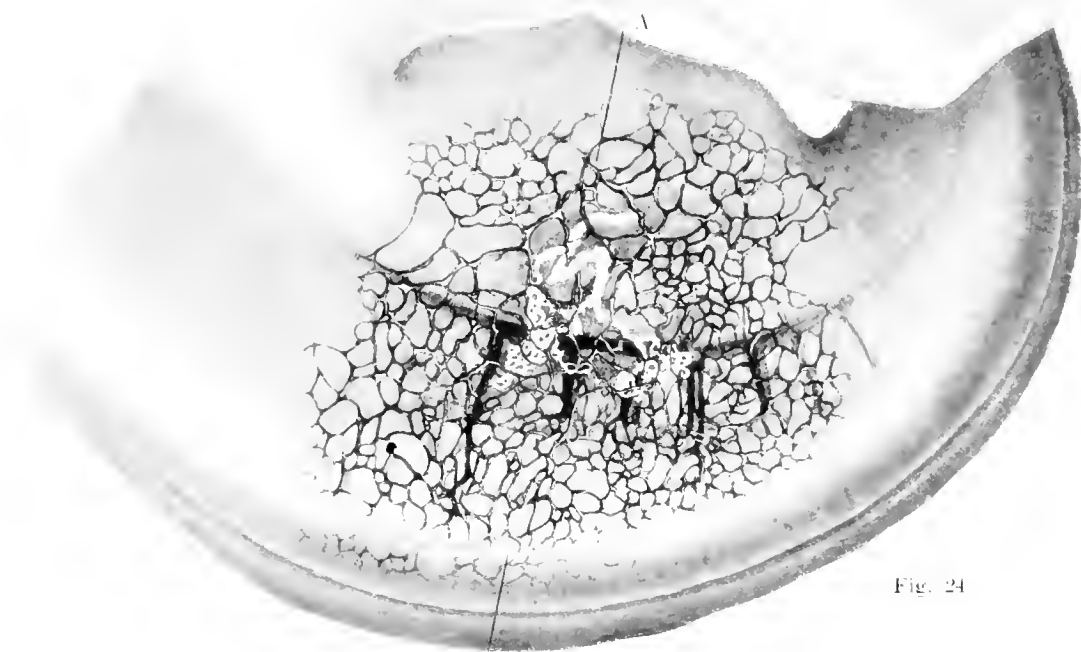


Fig. 24

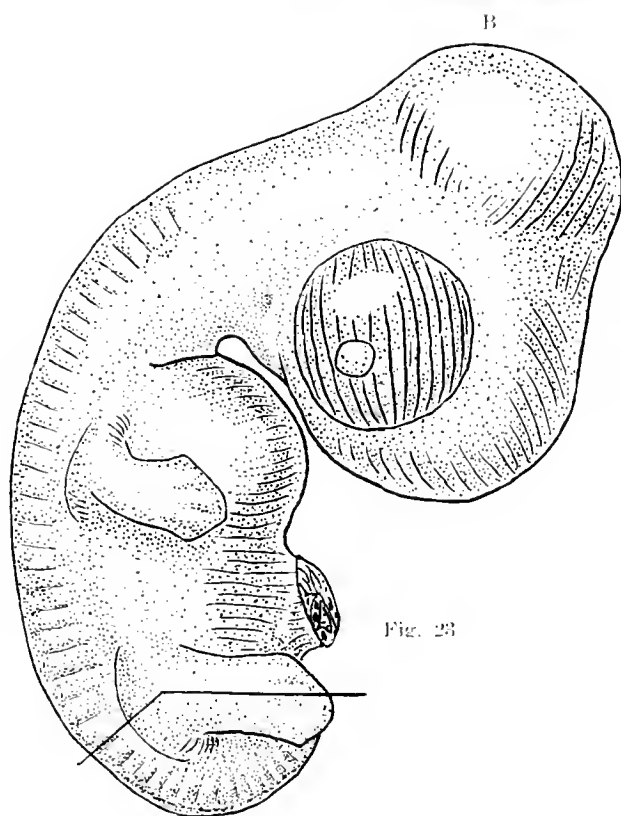


Fig. 23

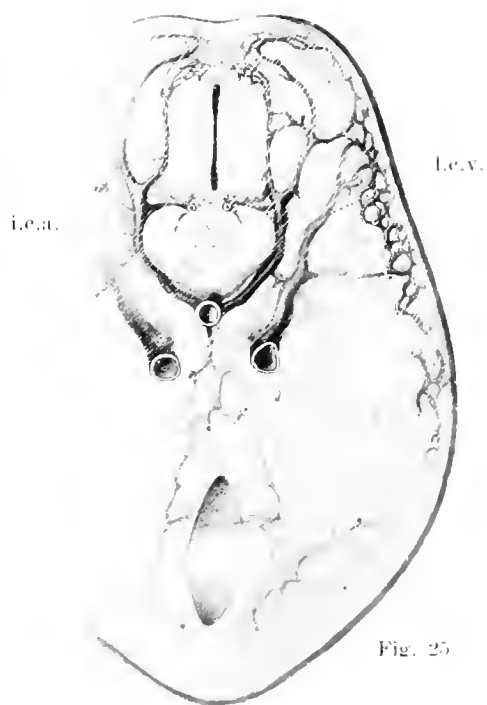


Fig. 25





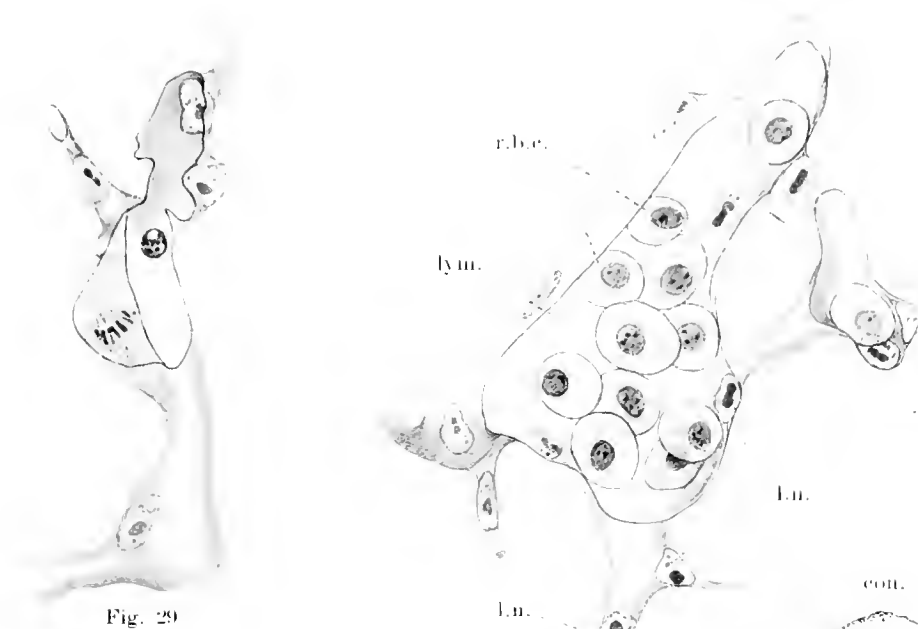


Fig. 29

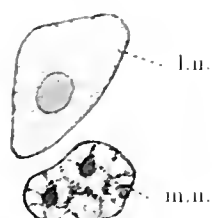


Fig. 27

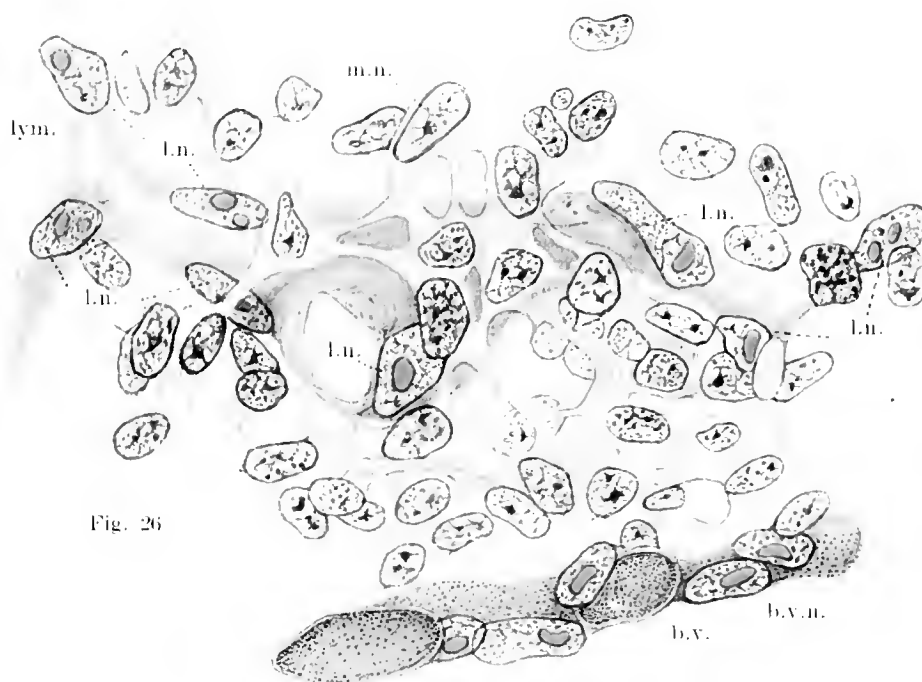


Fig. 26





Fig. 30



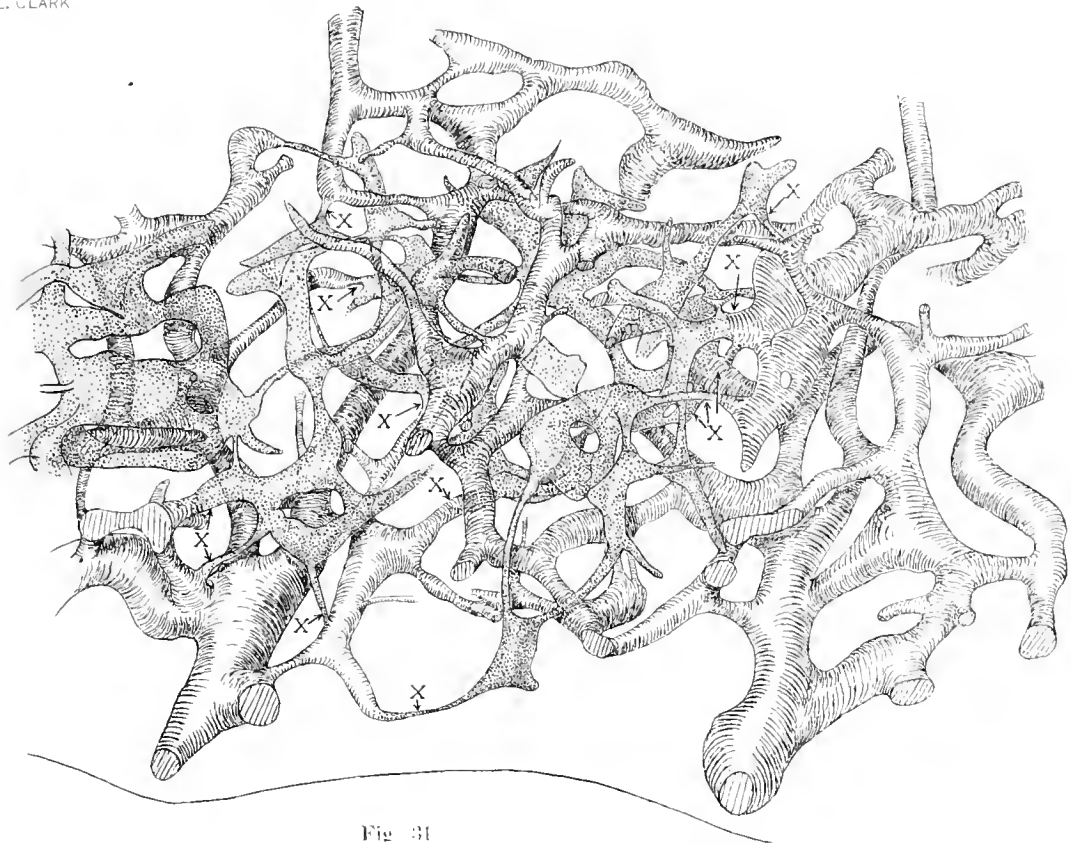


Fig. 31



Fig. 32



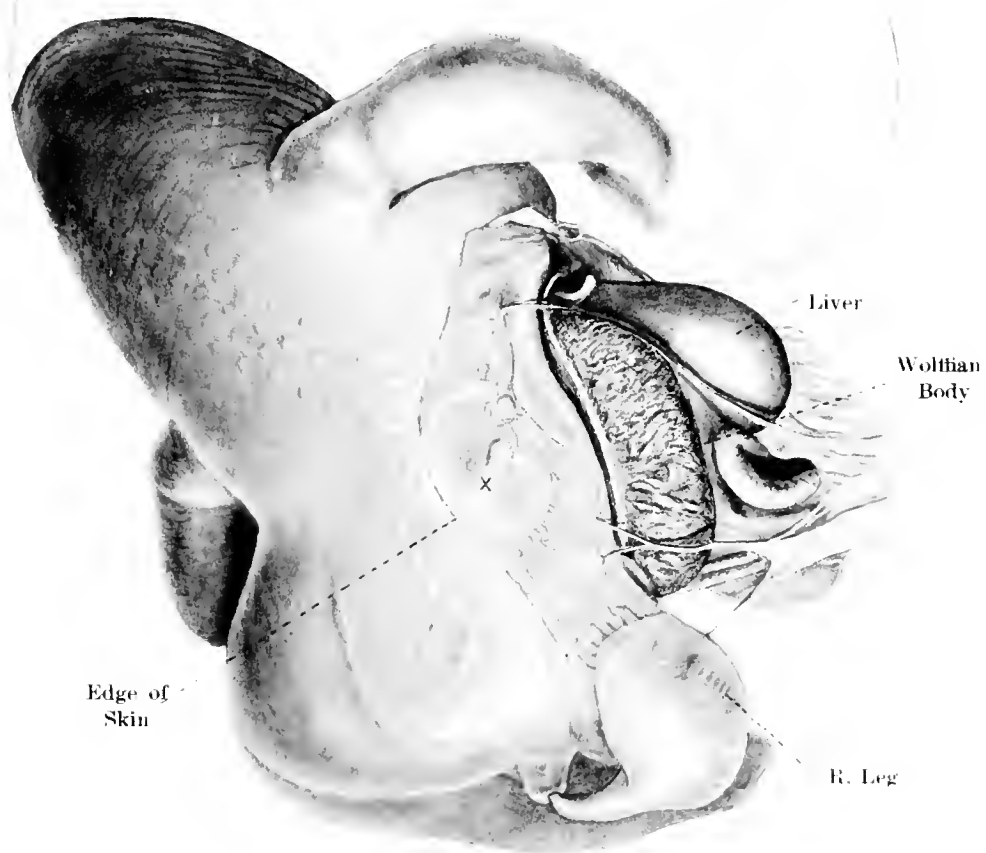


Fig. 33





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CONTRIBUTIONS TO EMBRYOLOGY, No. 46

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THE HEIGHT-WEIGHT INDEX OF BUILD IN RELATION TO LINEAR  
AND VOLUMETRIC PROPORTIONS AND SURFACE-AREA OF THE  
BODY DURING POST-NATAL DEVELOPMENT.

By C. R. BARDEEN,

*Professor of Anatomy in the University of Wisconsin.*

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With eleven charts and two text-figures.

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## CONTENTS.

	PAGE.		PAGE.
Introduction .....	485	Linear proportions— <i>continued</i>	
Volumetric Proportions .....	486	Breadths and depths— <i>continued</i>	
Specific gravity of the body .....	486	Breadth of head .....	514
Height-weight index of build .....	488	Breadth of neck .....	514
Alterations in volume .....	491	Bi-acromial breadth .....	515
Curves of volumetric proportions .....	493	Breadth of chest .....	515
Surface-area .....	495	Depth of chest .....	515
In relation to volume .....	495	Depth of abdomen .....	515
Relative regional distribution .....	498	Bi-crestal breadth .....	516
Linear proportions .....	500	Bi-trochanteric breadth .....	516
Grouping by age and by stature .....	502	Girths .....	516
Curves of linear proportions .....	504	Girth of head .....	516
Vertical measurements .....	506	Girth of neck .....	517
Vertex to external acoustic meatus .....	506	Girth of shoulders .....	517
Vertex to top of larynx .....	506	Girth of chest .....	517
Height of acromion .....	507	Girth of waist .....	517
Sitting-height .....	507	Girth of pelvis .....	517
Height of iliac crest .....	509	Girth of hips .....	518
Height of anterior superior iliac spine .....	509	Bone-girths .....	518
Top of pubic crest .....	509	Muscle-girths .....	518
Top of great trochanter .....	509	Growth and the height-weight index of build .....	519
Length of lower extremity .....	510	Height-weight-index growth-curve .....	519
Height of knee-joint .....	510	Weight-for-height curves .....	521
Height of ankle-joint .....	511	Curves for infancy .....	522
Segmental proportions of lower extremity ..	511	Curves for childhood .....	525
Length of foot .....	511	Curves for adolescence .....	527
Length of upper extremity .....	512	Curves for maturity .....	528
Length of arm .....	512	Individual growth-curves .....	531
Length of forearm .....	513	Transverse section and transverse diameter indices ..	532
Length of hand .....	513	Conclusions .....	538
Segmental proportions .....	513	Appendix I, tables A-L .....	542
Breadths and depths .....	514	Bibliography .....	553
Span .....	514		

# THE HEIGHT-WEIGHT INDEX OF BUILD IN RELATION TO LINEAR AND VOLUMETRIC PROPORTIONS AND SURFACE-AREA OF THE BODY DURING POST-NATAL DEVELOPMENT.

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BY C. R. BARDEEN.

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## INTRODUCTION.

The purpose of this paper is to illustrate the value of the height-weight ratio as an index of the proportions of the human body and to suggest the usefulness of an index of this character as an important factor in coordinating investigations in the field of gross human anatomy. In the study of human embryology it has become the custom to use the vertex-breach length as an index of stage of development. While not a perfect method, this index is of great value and has done much to coordinate the investigations of different observers and to aid in the formation of a science of early human growth. In studies of post-natal development the most commonly used, simple characteristics of differentiation of the body are sex, age, height, and weight. Even these simple characteristics are too frequently disregarded in anatomical studies, and there has accumulated a vast amount of descriptive and statistical material which it is impossible to coordinate because of this neglect. For a satisfactory science of human anatomy the data concerning human structure must be coordinated into an orderly system of knowledge regarding organic differentiation in relation to the life-cycle of varied types of human beings under varied conditions.

We can not make progress in the study of types without the use of common standards with which to compare studies made on different individuals or groups of individuals. For such standards to be established we need the general adoption of systematic methods of investigation. Methods of this kind have been adopted by anthropologists in the study of cranial and other measurements with success. Anatomists, however, have no uniform standards of recording the general characteristics of the development of the body in describing human structure. Data concerning race, sex, age, height, and weight (at least) should be furnished in connection with all investigations regarding the organization of the human body.

Race and sex furnish definite information, the importance of which we need not discuss. Age is of interest chiefly as a measure of the stage in the life-cycle reached by the individual at the time the study of his structure is made. As such it is imperfect, since some individuals pass more rapidly through the life-cycle than others, and the rapidity of development in individuals of the same essential life-cycle may vary greatly at different periods. Thus the studies of Roberts (1878) show that the children of the artisan classes in England grow more slowly than

those of the wealthier classes up to adolescence, but that during adolescence the former continue to grow for a longer period; so that, although the difference is not completely made up, there is at a given age less relative difference in size between adults of the two classes than between children of the two classes. The school children studied by Baldwin (1914) grew on an average much faster than those studied by other American observers up to adolescence, but the adolescents were of normal size. In table G, p. 596, data are given on rate of growth in American children.

Variation in rapidity of growth in stature and weight is, to an extent as yet incompletely determined, correlated with chronological variation in such processes as dentition (Boas and Wissler, 1904; Bean, 1914; ossification (Rotch, 1910), pubescence (Crampton, 1908), and the beginning of menstruation.<sup>1</sup> Crampton's observations support the view that there is a closer correlation between these processes and growth in height and in weight than there is between them and chronological age. In contradistinction to chronological age he uses the term *physiological age* to characterize a stage in which events connected with these processes occur. Weissenberg (1911) states that menstruating girls 13 years of age usually have the stature normal for 15-year old girls, while 15-year old girls who have not menstruated are usually of subnormal stature. It would doubtless be of great value to the study of human anatomy if anatomists would make a habit of recording, so far as practicable, the characteristics of the physiological age as well as the chronological age of the individual. Stratz (1915) has published a chart to illustrate ossification and dentition in relation to growth.

While study of the correlation of the development and structure of one part of the body with that of another part is of great value, the study of each part should be correlated with that of the size and general proportions of the body as a whole. Measurements of stature and of weight are the most common and the most important measurements of size. Linear measurements, other than those of stature, such as those of sitting-height or chest-girth, are of value in the study of bodily proportions but prove of greatest interest when viewed in relation to stature. Where practicable, it would be helpful to record the volume of the body as a whole, but the difficulties of technique exclude this as a routine procedure. Instead, we may estimate volume from weight. While such an estimate must necessarily be imperfect, since the specific gravity of the body as a whole varies, it adds considerable interest to the study of the size of the body to think of weight in terms of volume.

#### VOLUMETRIC PROPORTIONS.

The *specific gravity* of the different tissues of the body varies greatly. The data tabulated by Vierordt (1906) show that the densest tissue is the enamel of the teeth—2.380 (Davy), and next to this, bone—1.717 to 1.9304 (W. Krause and G. Fischer). On the other hand the air cavities in the head, including the nose, mouth, pharynx, and air sinuses, the air in the lungs and the gas in the stomach and intestines, all add to the volume of the body without increasing its weight. Most of the

<sup>1</sup> For the correlation existing between retardation and acceleration in growth and school grade the reader is referred to Porter (1893), MacDonald (1897-8), Boas and Wissler (1904), Smedley (1900), Crampton (1908), and Curtis (1917).

soft tissues of the body, including the blood, have a specific gravity in the neighborhood of 1.050. Fat is the least dense of these soft tissues. The human panniculus adiposus has been estimated to have a specific gravity of 0.971 (Kapff). Fibrous tissue is relatively heavy. The dermis of the human back has been estimated to have a specific gravity of 1.394 (Kapff) and tendons that of 1.1165 (W. Krause and G. Fischer). On the change in the specific gravity of the various tissues which may take place between infancy and maturity we have no good data.

The specific gravity of the human body as a whole has been investigated by a number of authors with somewhat divergent results. The highest figures cited by Vierordt are those of Krause, 1.0551 in quiet expiration, 1.1291 with air expelled from lungs and gas from alimentary canal. At the other extreme, Hermann gives a specific gravity of 0.9213 for normal cadavers, 0.9021 for individuals 11 to 20 years of age, and 0.9345 for individuals 21 to 40 years of age. The most accurate work appears to be that of Meeh (1879) and that of Mies (1899), who give somewhat similar figures. Meeh gives 1.01241 (extremes 0.978, 1.079) as the specific gravity of four children 6.70 to 13.125 years of age, and 1.028 (extremes 1.013, 1.057) for seven males 16 to 45 years of age, in expiration. Mies gives an individualized list of the boys and men whose specific gravity he determined. Apparently the specific gravity was taken during shallow respiration with the chest unexpanded. He summarizes the results as follows:

TABLE 1.

Number studied.	Classification.	Specific gravity.					
		Lower third.		Middle third.		Upper third.	
15	Boys.....	1.0123	to 1.023	1.024	to 1.029	1.030	to 1.048
59	Adults.....	1.0127	1.031	1.032	1.039	1.040	1.059
28	Convicts...	1.018	1.039	1.040	1.048	1.049	1.082

The convicts in general he states were thinner than the other adults whom he calls "ehrbaren" men. The specific gravity was determined, however, by different methods in the two series. A few examples of his individual cases may illustrate the relation of specific gravity to size of body.

TABLE 2.

Age.	Stature, centimeters.	Weight, kilos.	Index of build.		Specific gravity.
			Centim.-gram.	Inch.-pound.	
30	155.1	52.510	.0141	.508	1.034
30½	154.8	57.760	.0156	.562	1.036
22	160.2	62.830	.0153	.552	1.047
20½	159.6	51.780	.0135	.488	1.026
35	165.7	54.770	.0120	.435	1.035
61	165.8	98.09	.0215	.777	1.011
39	170.0	68.72	.0140	.505	1.042
27	179.0	68.24	.0119	.430	1.031
21	180.7	60.70	.0103	.371	1.019

Although in general fat individuals seem to have a relatively low specific gravity, one very thin individual (the last on the list) also has a low specific gravity. Under the limitations of the technique used the correlation between slightness of build and specific gravity does not seem close. In Meeh's studies of volume (table B, p. 543), the specific gravity of two new-born infant cadavers averages 1.047. Three males, with a trunk volume of 43.8 to 50.3 per cent of the total volume of the body, give an average specific gravity of 1.055; while five with a relative trunk volume of 50.8 to 56.6 per cent give an average specific gravity of 0.969. Of two young women, one, with a relative trunk volume of 50.0 per cent, gives a specific gravity of 1.006; the other, with a relative trunk volume of 49.7, gives a specific gravity of 1.016. The specific gravity of the body as a whole is thus greatly influenced by the amount of expansion of the thorax. According to Ziegelroth (1896), the difference between the volume in expiration and that in inspiration in the adult may be over 3 liters.

There is no evidence that the specific gravity of the adult body differs greatly from that of the infant, although the greater relative amount of water in the infant's body and the relatively small amount of bone might lead one to infer that the infant's body would have a lower specific gravity. There is probably more gas normally present in the adult body. Donaldson (1903) cites a table from E. Bischoff, in which it is shown that while the relative weight of the skeleton varies little from infancy to maturity, that of the muscles nearly doubles, that of the thoracic and abdominal viscera is decreased by one-third, that of the brain is decreased to one-sixth to one-eighth of the infantile proportions, that of the skin to one-half, and that of the fat is greater in the adult female than in the infant, less in the adult male than in the infant.

The volume of a pound of water is approximately 27.68 cubic inches at 4° C. At 37° C. (98.5° F.) it is 27.862 cubic inches. The specific gravity of a 3-inch cube (27 cubic inches) weighing a pound would therefore be 1.0252 at 4° C. or 1.032 at the temperature of the body. These last figures approximate the specific gravity of the body, during quiet respiration, chest unexpanded. It is therefore convenient to consider a pound of the human body as equivalent to a 3-inch cube. A pound occupies less space than this in regions like the hand, where the tissue has relatively high density, and more in regions containing gas. The estimate is sufficiently accurate for general purposes. If desired, allowance may be made for variations in specific gravity in different parts of the body, or in individuals of different build.

*Height-weight index of build.*—The volumes of objects of the same shape but of different sizes vary as the cube of a given diameter through these objects. The volumes of the bodies of individuals of the same external form but of varying heights vary as the cube of the stature multiplied by a factor which is conditioned by the form of the body, as  $\pi$  is conditioned by the form of a sphere. This factor, in the case of the relation of volume to stature, expresses the part of a space equal to the cube of the height occupied by the volume of the body. Thus, if we assume that 27 inches is the volume of a pound, the volume of a man weighing 150 pounds

would be 4050 cubic inches. If he were 68 inches tall the volume of his body would occupy  $\frac{4050}{314432}$  of a space equal to the cube of his height or, expressed in terms of percentage, 1.288 per cent. The body of an individual 50 inches tall and of the same shape would occupy the same proportions of the cube of his height, or 1.288 per cent of 125,000, 1610 cubic inches. At 27 inches to the pound this would mean a weight of 59.6 pounds. Similarly, an individual 20 inches high of the same form would have a volume of 103.04 cubic inches, and a weight of 3.81 pounds. Conversely, if the ratio of volume of the body to the cube of the height differs in two individuals the form of the body of the two individuals must differ. Thus it is clear that if the volume of the body of one individual occupies a greater part of the space equal to the cube of his height than is the case with another individual, the first individual must have a relatively greater transverse section. If a new-born infant 20 inches long weighs 7.34 pounds, assuming that a pound is equal to 27 cubic inches, his volume would be 198.18 cubic inches. This volume would occupy 2.477 per cent of a space equal to the cube of his height, or nearly twice as much as if he had the form of the 150-pound man referred to above. His relative transverse cross-section would therefore be about twice as great. By correlating this difference of ratio with difference in body form we are enabled to use the ratio as an index of specific differences of form in so far as the correlation holds. Since individuals do not develop uniformly and vary in body form at all stages of development, the correlations of the ratio above mentioned with body form can be at best merely approximate, but with its limitations it is of great value as an expression of build.

For practical purposes it is more convenient to divide the weight directly by the cube of the height and to use the product as the height-weight coefficient or index of build, rather than to estimate volume from the weight and use the ratio described above. On the assumption that a pound of human body occupies 27 cubic inches of space, the height-weight index is  $\frac{1}{27}$  of this ratio. Thus a man 68 inches tall, weighing 150 pounds, would have a height-weight index of  $\frac{150}{314432}$  or 0.000477, which is  $\frac{1}{27}$  of the volume-cube of height ratio—0.01288. Similarly, the infant 20 inches long, weighing 7.34 pounds, has a height-weight index of 0.0009175 or  $\frac{1}{27}$  of the volume-cube of height ratio—0.024773. Since the indices just described are inconvenient to use freely, owing to the position of the decimal point, we may multiply each index by 1,000 and thus express it in terms of thousandths or of percentages. Thus the index 0.000477 becomes 0.477 or 47.7 per cent, a form of expression easy to remember and understand. We reach the same result by dividing the weight in pounds by the cube of a tenth of the height or by the thousandth part of the cube of the height in inches. Therefore, as a height-weight index in the study of stature, weight, and body-form, we have adopted the weight of the body in pounds divided by the thousandth part of the cube of the height in inches. Using this index, we find infants during the first half of the first year after birth usually have an index in the neighborhood of 0.918; that is, the weight of the body usually approx-

imates 91.8 per cent of the thousandth part of the cube of the stature. In an adult male 68 inches tall it usually approximates 47.8 per cent at 30 to 35 years of age.

We have used the inch-pound units because a large part of the best statistical studies on height and weight have been made in England and America with the use of these units. For the sake of uniformity in this article we have made use of the inch-pound height-weight index even when dealing with material expressed in metric system units. In general, however, in dealing with the latter it is best to use

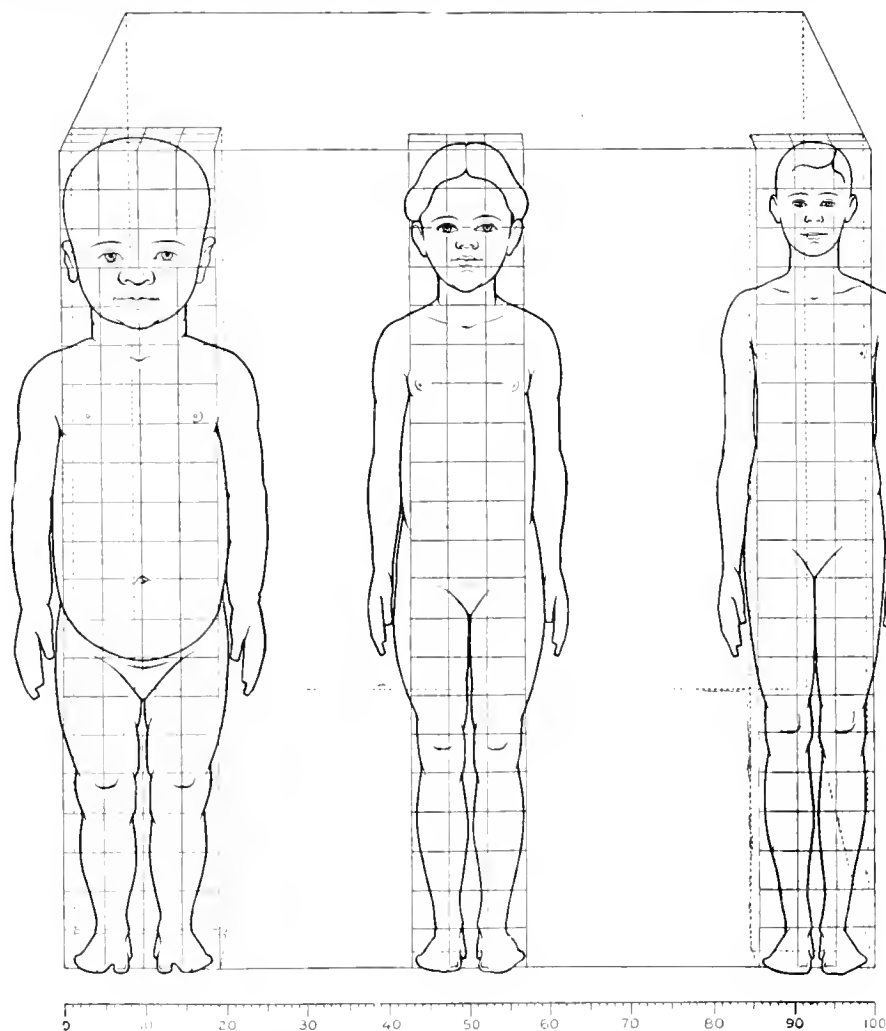


FIG. 1.—Diagram to illustrate the proportions of the body relative to the cube of the height in infancy, childhood, and adolescence. The infant is 21 inches long, the child 42 inches long, and the youth 63 inches long. For further description see text, p. 491.

a height-weight index based directly on these units. If we divide the weight in grams by the cube of the height in centimeters we obtain an index which has 2.768 per cent of the value of the inch-pound index here adopted. To reduce the inch-pound index to the centimeter-gram index multiply the former by 0.02768. To change the centimeter-gram index to the inch-pound index either divide it by 0.02768 or multiply it by 36.13. Rohrer, in 1908, clearly pointed out the value of the quotient obtained by dividing the weight in grams  $\times 100$  by the cube of the



height in centimeters as an index of "Körperfülle." Its value has been recognized by Martin in his *Anthropologie* (1914). It has, however, been but comparatively little used. Bobbitt (1909) makes use of the reciprocal index and divides the cube of the height by the weight. In tables D and E both the inch-pound and the centimeter-gram units are used to illustrate the ratio of weight to height in connection with the data of Weissenberg and of Quetelet on linear proportions.

*Alterations in volume.*—The relation of volume to the cube of the stature during three periods of development (infancy, childhood, and adolescence) and the associated proportions of the body are illustrated in figure 1. The figure at the left is that of an infant about 2 weeks old, 21 inches long, weighing 8.5 pounds. The middle figure is that of a well-developed child 5 years old, 42 inches long, weighing 39.3 pounds. The figure at the right is that of a youth 15 years old, 63 inches tall, weighing 104.5 pounds. The figure of the infant is placed within an oblong block 21 inches high, 4 inches wide, and 3 inches deep. Each of the smaller squares on the surface of the block represents a square inch. The volume of the infant is estimated, at 27 cubic inches to the pound, to be 229.5 cubic inches. The volume of the block in which it stands is 252 cubic inches. The ratio of volume of infant to volume of cube of stature is  $\frac{229.5}{9261}=0.02478$ . The height-weight index is  $\frac{8.5}{9.261}=0.9178$ .

The figure of the child is at twice the scale of that of the infant. It is placed within an oblong block 42 inches high, 6 inches wide, and 5 inches deep. Each of the smaller squares on its surface represents 4 square inches, or a cube of 8 cubic inches. The volume of the oblong block is 1260 cubic inches. The volume of the child's body is 1061.1 cubic inches. The volume of the cube of the height is 74,088 cubic inches. The ratio of the volume of the child's body to the cube of its stature is  $\frac{1061.1}{74088}=0.014322$ . The height-weight index is  $\frac{39.3}{74.088}=0.53045$ .

The figure of the youth is at three times the scale of that of the infant. It is placed within an oblong block 63 inches high, 9 inches wide, and 5 inches deep. Each of the smaller squares on its surface represents 9 square inches or 27 cubic inches, *i. e.*, one "flesh" pound. The volume of the oblong block is 2835 cubic inches. The volume of the youth's body is 2821.5 cubic inches. The volume of the cube of the stature is 250,047 cubic inches. The ratio of the volume of the youth's body to the cube of the height is  $\frac{2821.5}{250047}=0.011284$ . The height-weight index is  $\frac{104.5}{250.047}=0.418$ .

On comparing the height-weight indices of the three figures we see that that of the infant, 0.918, is nearly 75 per cent greater than that of the child, 0.530; while that of the child is only about 25 per cent greater than that of the youth. It is also obvious that the figure of the child much more nearly resembles that of the youth than that of the infant. The changes in the relations of volume to cube of the height are associated with changes in the proportions of the body, which are far greater during the first 5 years after birth while the child is growing 21 inches in height than during the next 10 years when he grows another 21 inches in height. The latter are, however, in the same direction in both cases, and the most striking features are the decrease in the relative volume of the head and the relative increase in the relative volume of the inferior extremities. The relative volumes of the trunk and upper extremities change comparatively little. The relative volumes of the

head to the level of the larynx, of the trunk below the level of the larynx, of the lower extremities to the crotch, and of the upper extremities, are estimated as follows in each of the three individuals:

TABLE 3.

	Infant.	Child.	Youth.
Stature.....	21 inches.	42 inches.	63 inches.
Weight.....	8.5 lbs.	39.3 lbs.	101.5 lbs.
Index of build.....	0.918	0.530	0.418
Estimated volume.....	229.5 cu. in.	1061.1 cu. in.	2821.5 cu. in.
Relative volume of—			
Head.....	0.280	0.164	0.082
Trunk.....	0.495	0.523	0.523
Lower extremities...	0.135	0.226	0.292
Upper extremities...	0.090	0.087	0.103
	1.000	1.000	1.000

Subsequent to the stage represented by the figure of the youth, the growth in stature of the average American adolescent is 4.5 to 5 inches and usually full stature is nearly reached before the age of 20. The average American woman does not exceed in stature that shown by the youth. At 15 she is usually an inch shorter and reaches full height about 2 years before the man. For some years in both sexes after full stature is reached the body continues to increase in girth, through muscular development. Subsequently it usually increases through adiposity. In old age there is decrease in stature, which may amount to 3 cm. or more at age 70 and is more marked in tall than in short individuals, and in women than in men (Manouvrier, 1902). This decrease in stature is said to be due mainly to loss in elasticity in the intervertebral disks. The lower extremities become relatively long. There is also usually a retrograde metamorphosis in the musculature. Figure 2 represents the proportions of the body of a man and of a woman, each about the age of 30, of average stature and weight, and of an old man 60 to 70 years of age. The proportions of maturity and old age may thus be compared with the proportions of the body in infancy, childhood, and early adolescence. From the standpoint of relative volume these proportions may be expressed as follows:

TABLE 4.

	Mature man.	Mature woman.	Old man.
Stature.....	67.5 inches.	63 inches.	67.0 inches.
Weight.....	148 lbs.	127.5 lbs.	155 lbs.
Index of build.....	0.481	0.510	0.515
Estimated volume.....	3996 cu. in.	3442.50 cu. in.	4185 cu. in.
Relative volume of—			
Head.....	0.071	0.068	0.070
Trunk.....	0.542	0.517	0.573
Lower extremities...	0.285	0.320	0.265
Upper extremities...	0.102	0.095	0.092
	1.000	1.000	1.000
Volume of block in which each figure stands.....	3996 cu. in.	3440 cu. in.	4188 cu. in.
Cross-section.....	59.2 sq. in.	54.6 sq. in.	62.5 sq. in.

These estimates of volume of the various parts of the body are based primarily on the data published by Meeh (1895), see tables B and C. These data have been supplemented by a few observations of my own on the volume of the cadavers of an infant and a young child, the volume of a living child of 6 years, and the volumes of the lower extremities in 20 young men; as well as by a study by one of my students, K. L. Puestow, of the volume of the head in 34 adults and 6 children. The data are too scanty to furnish more than approximate estimates of normal relative volume.

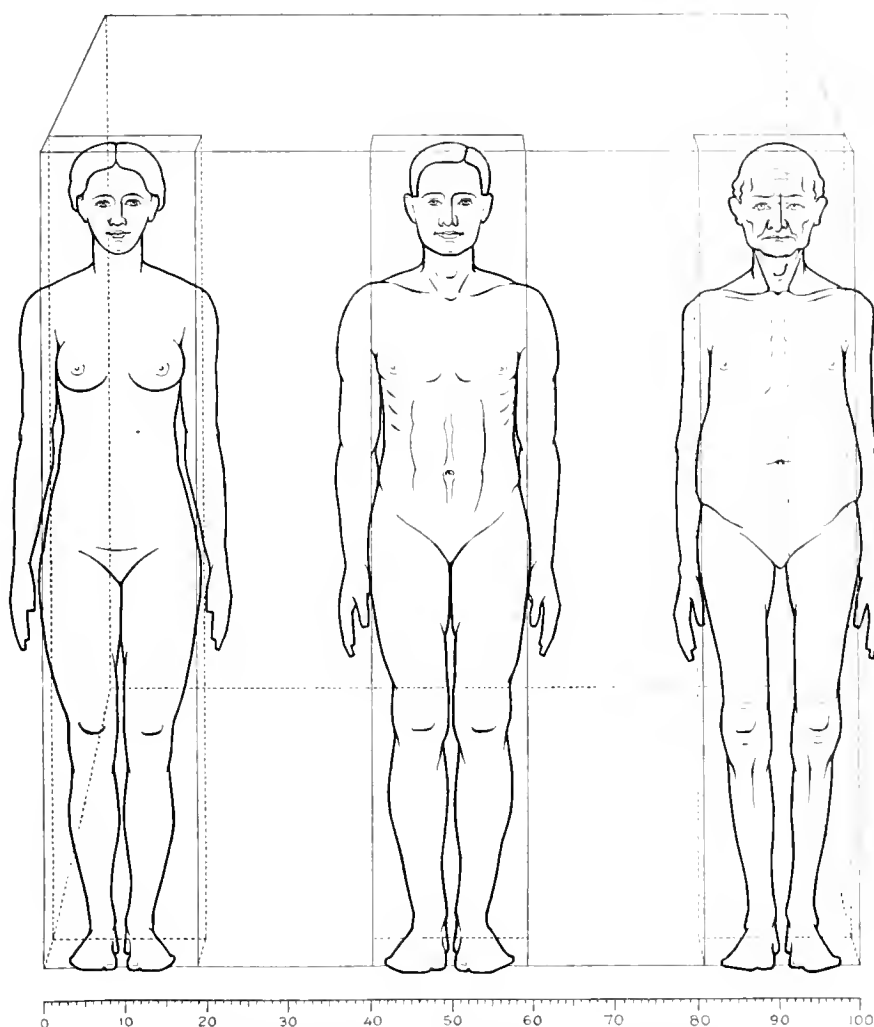


FIG. 2.—To illustrate the proportions of the body of a man and of a woman, age about 30, of average height and weight, and the body of a man of 60 to 70 years. See text, p. 492.

*Curves of volumetric proportions.*—I have endeavored in chart K, p. 538, to illustrate by curves the changes in relative volumetric proportions which take place during growth in individuals of normal build. The figures from which these curves are plotted are given in table K. It is assumed that the distance from the top to the bottom of the chart represents total volume at any given stature. This total volume is subdivided into the volume of the head to the larynx, that of the trunk from the top of the larynx to the crotch, that of the inferior free extremities (sub-

divided into thighs, legs, and feet), and that of the upper extremities (subdivided into arms, forearms, and hands). Curved lines show the alteration in the relative volumetric proportions as we pass from a stature of 20 inches at the left to one of 75 inches at the right. A curve of the height-weight index of build has been inserted in the chart to show the course of development of the average American male who reaches a stature of 67.5 to 68 inches. The part of a similar curve for the average female where it departs markedly from that of the male is shown by broken lines. Curves for volume are likewise shown by broken lines where the proportions of the female body diverge markedly from those of the male. The ages given at the bottom of the chart represent the approximate ages at which average Americans reach the stature given at the top of the chart. It will be noted that there is a marked dip in the curve separating trunk volume from the volume of the lower extremities and that the center of this dip lies at the line of average adult male stature. This dip represents the change in relative bodily proportions which takes place during adolescence, an increase in the size of the trunk marked by a relatively greater growth in length than that which takes place in the lower extremities, and by even greater relative increase in girth. In a group of individuals reaching less than average American stature this dip shifts to the left. In a group of more than average stature it shifts to the right and is less marked. The older the group of individuals studied the deeper the dip up to 50 years of age. In women the lower extremities from the calf up are relatively greater in girth and in volume than in men and take a greater part in the relative lateral expansion which marks adolescence and early maturity. We have no data on changes in relative volumetric proportions of trunk and lower extremities corresponding to those in men, but if it occurs it is much less marked. The studies of the volume of the lower extremities of the 20 young men, 21 to 25 years of age, referred to above may be summarized as follows:

TABLE 5.

No. of individuals.	Average stature.	Average weight.	Average index of build.	Relative volume.			
				Lower extremities.	Thigh.	Leg.	Foot.
6	<i>inches.</i> 65.9	<i>pounds.</i> 142.5	0.498	0.283	0.797	0.467	0.150
4	67.8	151.0	.484	.284	.788	.480	.150
3	69.3	145.0	.436	.305	.904	.464	.156
3	70	154.0	.449	.319	.954	.493	.147
4	71	158.0	.441	.310	.903	.487	.161

The volume of the body is estimated from the weight, the specific gravity being assumed to be 1.0252. The relative volumes of the lower extremities and their subdivisions are expressed in relation to volume=1. The volumes of the thighs, legs, and feet of each individual are averaged. The volume of each extremity was measured to the level of the crotch. Meeh likewise measured the volume of the lower extremities to the level of the crotch and this is the level represented in chart K. Harless, in weighing the lower extremities, made a cut which included the great trochanters. This must be taken into account in the use of table C. Meeh's data

(see table B) show that the volume of the lower extremities is probably relatively greatest in the period preceding puberty, at the time when their length is relatively greatest. The data given above show that tall men have relative volumetric proportions of the lower extremities which resemble those of boys in the stage preceding puberty.

Meeh gives figures for neck-volume which vary considerably for different individuals, probably due mainly to difficulties in the technique used. It seems best to measure the neck above the level of the larynx as a part of the head, below this level as a part of the trunk. In making use of Meeh's data in constructing the line which represents the division between trunk-volume and head-volume in chart K we have transferred about 1 per cent of the total volume from his neck-volume to head-volume and put the rest with the trunk-volume. These data indicate that the relative volume of the head and of the upper extremities is smaller in women than in men. In fat adults the absolute volume of the head measured as above suggested is considerably increased by the "double chin," but the main relative increase usually comes in the trunk (see table B, Meeh's case No. 4, with a height-weight index of 0.610).

C. D. Spivak (1915) gives some figures on head-volume which show great irregularity in relative head-volume. In general the relative head-volume is low compared with that shown in chart K, even when allowance is made for relative adiposity. Spivak also gives data on volume of the body to the level of the umbilicus, to the level of the nipple, and to the level of the larynx, and on specific gravity. The work of Mr. Puestow has not yet been completed, but his data correspond closely with those of Meeh described above and have proved of value in the construction of the curve for volume of the head. They show furthermore that relative to the volume of the body the volume of the cranial portion of the head is more reduced than the facial portion as the stature increases.

Further detailed estimates of relative volume are given in tables B, C, and K and in table 7 in the following section.

#### SURFACE-AREA.

The *relation of cutaneous surface to volume* of the body is one of considerable physiological interest. Surface-area is conditioned by shape and by volume. For a given volume the sphere has the smallest surface-area. Of elongated bodies of constant cross-section the cylinder has the smallest surface-area. In the human body the head is spherical, the neck and limbs are cylindrical, and the trunk may be compared to a flattened cylinder which tends to the spherical in fetal life, in infancy, and in the fat adult. Du Bois and Du Bois (1915) have proposed an ingenious method of estimating surface-area from certain lengths and girths of each of the parts mentioned. The neck is included with the trunk. The constants used in estimating surface-area of each part, corresponding to  $\pi$  in treating of spheres and cylinders, have been determined empirically by observations on the cutaneous areas of a widely diverse but limited numbers of individuals. Benedict (1916) has proposed a method of estimating the surface-area of the body from silhouette photo-

graphs taken with the body in definite postures. The constant which he used to estimate body surface from silhouette area is based on the Du Bois observations on surface-area. The Benedict method has the great advantage of furnishing useful records of body shape.

Neither of the methods mentioned are based on data concerning volume or on weight as an index of volume. On the other hand, Meeh (1879), to whom we are still indebted for the most extensive series yet made of careful observations of surface-area of normal individuals during growth, proposed a method of estimating surface from weight by the use of the formula  $S = KW^{2/3}$ , in which  $S$  = area,  $W$  = weight, and  $K$  is a constant based on Meeh's observed data. The disadvantage of this method lies in the fact that it can apply with a given constant only so far as the individuals whose surface is estimated are similar in shape. For infants  $K$  is smaller than for adults. Several methods have been proposed for lessening the disadvantage mentioned by means of the use of various linear measurements in constructing a formula for estimating surface. For a brief review of these methods the reader is referred to Du Bois and Du Bois (1915). None has proved to be of much practical value.

Of the linear measurements of the body, stature in relation to weight gives us, as we have seen, the most practical simple index of shape. For estimating surface-area, therefore, it is of value to use the ratio between observed surface-area and the surface of an object which has the same length as the stature of the body and a volume which may be estimated from body-weight. For the purpose proposed it is simplest to assume a specific gravity of 1.000 if we are dealing with metric-system units or of 1.0252 if we are dealing with inch-pound units. In the former case we divide the weight in grams by the height in centimeters. The quotient gives us the average cross-section in square centimeters of an object as long as the body and of the same volume. This elongated object may be conceived either as circular or as square in cross-section. In the latter case the surface of the object is larger than in the former. If we assume the object to be a flattened block, as we have in considering volume, the surface becomes still larger. I have chosen the block with a square cross-section as the simplest object for our present purpose. The formula for surface-area thus becomes:

$$S = K \left( 2 \frac{W}{H} + 4H \sqrt{\frac{W}{H}} \right)$$

where  $S$  = surface-area,  $W$  is weight in grams,  $H$  height in centimeters, and  $K$  is a constant. In the formula  $\frac{W}{H}$  gives the surface-area of each end of the block,  $4H \sqrt{\frac{W}{H}}$  the surface-area of one side of the block.  $K$  has to be determined from the observed surface-area of individuals of given height and weight. If inch-pound units are used, one must substitute  $W \times 27.68$  for  $W$  in the formula given above if the same specific gravity is assumed as in this formula, or  $W \times 27$  if one assumes the same specific gravity I have assumed in dealing with volume.

The value of  $K$  depends on the data one chooses as representing the most accurate observations on surface-area. The best observations appear to be those

of Meeh (1879), and those of Du Bois and Du Bois (1915), and of Sawyer, Stone, and Du Bois (1916). These we may refer to, respectively, as the Meeh and the Du Bois data. The Meeh data show an average value of about 15 per cent above the Du Bois data. One is therefore obliged to choose between the two, since the difference appears to be due to a consistent difference in results due to variation in methods. As I shall point out below, the Meeh data show a slightly greater value for head-surface, a slightly less value for the surface of the upper extremities than the Du Bois, but these differences are relatively insignificant and the difference in values in the two sets of data must lie in the general methods used.

The Du Bois data give an average value for  $K$  of 1.237 if we omit the data on an infant cadaver and a case considered by the authors of doubtful value. The extremes are 1.15 for a very fat woman and 1.29 for a tall, thin man. Using 1.237 as the value for  $K$ , and calculating area according to the formula given above, we get a series of estimated areas which in 2 out of 8 of the Du Bois cases are closer to observation than are his estimates based on linear measurements and in 6 are not so close. The differences between the two sets of estimates are slight except in case of one excessively fat woman for whom the difference amounts to 5 per cent of the surface-area. For the 2 normal men  $K$  has a value of 1.225, for the normal woman 1.256. For 3 thin men the average value is 1.275. In the Meeh cases cited by Du Bois and Du Bois,  $K$  has an average value of 1.452. If we take the entire series of Meeh's cases, including infants, and with them the case of Hecker, as Vierordt does, we find an average value for  $K$  of 1.444 and a mean value of 1.425. Taking  $K$  as 1.425, and using Meeh's data and the data of Hecker on stature and weight, we get a closer fit to the observed data than we do by the use of the Meeh formulæ for children and adults in 9 out of 17 cases, a less close fit in 5 cases, and about the same fit in 3 cases.

Estimating build from height-weight index, we find a value for  $K$  of 1.467 for thin individuals, of 1.454 for individuals of normal build, and of 1.394 for fat individuals. For the 6-day, 6-month, and year-old infants,  $K$  has the value respectively of 1.495, 1.465, and 1.53. For 5 children 2.75 to 13.12 years of age,  $K$  has an average value of 1.408 (extremes 1.38 to 1.43). For two boys 16 and 18 years of age it has a value respectively of 1.537 and 1.485. The 16-year-old boy has a metric height-weight index of only 0.01009 (inch-pound index 0.365). For 7 adults the average value of  $K$  is 1.43. The extremes are 1.36 in a fat individual 171 cm. tall with a metric height-weight index of 0.01565 (inch-pound 0.565) and 1.515 in a slender individual 158 cm. tall with a metric height-weight index of 0.01268 (inch-pound 0.458). For women Meeh gives no data. The one woman of normal build studied by Du Bois shows a relatively higher value, 1.255, than that of his men of normal build, 1.225. If we adopt Meeh's observed data and desire to make use of the formula suggested above, we are justified in taking 1.50 for  $K$  during infancy, 1.40 during childhood, 1.50 for the period of rapid growth in stature during adolescence, 1.42 for the average male adult, 1.45+ for tall, thin adults, and 1.40- for short, stout adults. It is probable that 1.45 gives an equivalent value for women of normal build.

Harris and Benedict (1919), in a publication on metabolism now in press, the proof sheets of which have been kindly sent me, give an extensive discussion of available data on surface area. They point out two sources of error in the Meeh formula, variation in body form and variation in specific gravity. From the standpoint of prediction of metabolism, the Du Bois height-weight chart was found to have the greatest value, next came Meeh's formulæ, and finally body-weight. Prediction from two direct measurements *stature* and *body-weight* give, however, more accurate results than the method of calculation from surface-area by the Du Bois height-weight chart. Still greater accuracy is obtained by including an age factor.

We have referred above to the work of Benedict (1916) on estimation of cutaneous area from silhouette area. He gives data on height and weight for each of the 20 cases studied. By use of 1.275 for  $K$  in the formula I have given above one can obtain values for surface-area which are practically as close to the Du Bois data as the values obtained by Benedict from his silhouette method. For females  $K=1.295$  gives closer results than  $K=1.275$ .

The use of the formula here proposed, based on height and weight, therefore gives results closer to observed data than the Meeh formula and nearly if not quite as close as the more elaborate linear and silhouette methods of Du Bois and Du Bois and of Benedict. Du Bois and Du Bois (1916) give another formula for estimating surface-area from height and weight and a chart based on this formula.

The *relative regional distribution* of the surface-area may be illustrated by means of data from Meeh (1879), Du Bois and Du Bois (1915), and Sawyer, Stone, and Du Bois (1916). Meeh gives data on regional cutaneous area in the 16 cases he studied, the other investigators in all of their cases. So far as I am aware, no investigator has studied regional distribution of surface-area in relation to the volume of the various parts of the body. It is therefore of interest to compare the relative volume of individuals studied by Meeh for volume (1895) with individuals of similar bodily proportions studied for surface-area. I have selected the following individuals to illustrate the relations of relative regional distribution to total surface-area and relative surface-area to relative volume:

TABLE 6.

No.	Author.	Designation.	Age.	Sex.	Stature, cm.	Weight, kg.	Index of build.		Total surface- area or volume.
							Centim.- gram.	Inch- pound.	
1	Meeh....	Infant.....	6 day...	Male.	50.0	3.02	0.0242	0.873	2504.8 sq. cm.
2	Meeh....	Cadaver....	Infant..	Male.	55.0	3.96	.0238	.860	3,757 liters.
3	Du Bois..	Anna M....	21 mo..	Fem..	73.2	6.27	.0160	.578	3699.0 sq. cm.
4	Meeh....	Cadaver....	22 mo..	Male.	77.0	6.83	.0150	.512	6,626 liters.
5	Du Bois..	Mrs. K.....	.....	Fem..	149.7	93.0	.0277	1.002	18592.0 sq. cm.
6	Meeh....	No. 9.....	16 yr...	Fem..	154.5	54.55	.0148	.534	54.21 liters.
7	Du Bois..	Morris S....	21 yr...	Male.	164.3	64.0	.0144	.521	16720.0 sq. cm.
8	Meeh....	No. 5.....	22 yr...	Male.	162.0	54.7	.0129	.465	52.87 liters.
9	Du Bois..	Emma W....	26 yr...	Fem..	164.8	57.62	.0129	.466	16451.0 sq. cm.
10	Meeh....	No. 10.....	22 yr...	Fem..	156.0	60.23	.0159	.573	59.31 liters.
11	Du Bois..	Gerald S....	18 yr...	Male.	171.8	45.25	.0089	.322	14772 sq. cm.
12	Meeh....	Naser.....	20 yr...	Male.	170.0	59.5	.0121	.437	18695.3 sq. cm.
13	Meeh....	No. 1.....	42 yr...	Male.	169.4	68.3	.0141	.508	67.22 liters.
14	Meeh....	Kehrer.....	36 yr...	Male.	171.0	78.25	.0157	.565	22434.9 sq. cm.
15	Du Bois..	R. H. H....	22 yr...	Male.	178.0	64.1	.0114	.410	18375.0 sq. cm.
16	Du Bois..	E. F. D. B..	32 yr...	Male.	179.2	74.05	.0129	.466	19000.0 sq. cm.



The relative surface-area and relative volumes of the chief regions of the body in the cases cited may be expressed in percentage of total area and total volume as follows:

TABLE 7.

No.	Stature, cm.	Index of build (centim.-gram).	Sex.	Head, relative.		Trunk, relative.		Lower extremities, relative.		Upper extremities, relative.	
				Surface.	Volume.	Surface.	Volume.	Surface.	Volume.	Surface.	Volume.
1	50.0	0.0242	Male...	18.2	.....	36.6	.....	24.8	.....	20.1	.....
2	55.0	.0238	Male...	.....	26.7	.....	49.3	.....	15.2	.....	8.8
3	73.2	.0160	Fem...	16.4	.....	36.6	.....	28.0	.....	19.0	.....
4	77.0	.0150	Male...	.....	26.4	.....	51.4	.....	14.7	.....	7.5
5	149.7	.0277	Fem...	5.9	.....	41.7	.....	36.5	.....	15.9	.....
6	154.5	.0148	Fem...	.....	7.0	.....	51.8	.....	31.7	.....	9.5
7	164.3	.0144	Male...	6.2	.....	37.7	.....	36.9	.....	19.2	.....
8	162.0	.0129	Male...	.....	7.9	.....	50.0	.....	30.0	.....	12.0
9	164.8	.0129	Fem...	6.6	.....	34.15	.....	40.7	.....	18.6	.....
10	156.0	.0159	Fem...	.....	6.3	.....	52.1	.....	32.4	.....	9.2
11	171.8	.0089	Male...	6.4	.....	33.9	.....	40.1	.....	19.6	.....
12	170.0	.0121	Male...	7.7	.....	34.4	.....	40.5	.....	17.5	.....
13	169.4	.0141	Male...	.....	7.4	.....	54.4	.....	28.0	.....	10.2
14	171.0	.0157	Male...	7.2	.....	39.7	.....	35.2	.....	17.8	.....
15	178.0	.0114	Male...	6.4	.....	35.1	.....	39.5	.....	19.0	.....
16	179.2	.0129	Male...	6.1	.....	34.6	.....	40.1	.....	19.2	.....

A study of this table will show that the chief differences in relative surface-area as estimated by the Du Bois method and by Meeh are a slightly greater head-surface area and a slightly smaller upper extremity surface-area by the former. Making allowance for this and remembering that a low index of build means relative thinness, a high index means large bulk, the following points seem clear:

The volume of the trunk including the neck is approximately 50 per cent of the total volume of the body throughout life; the surface-area is approximately 37 per cent of the total surface-area; the ratio of area to volume is approximately 3:4. Both volume and surface-area of the trunk are relatively greater in fat than in thin individuals and in men than in women. They are also probably greater in short than in tall adults, although this point as regards volume is not well brought out in this table.

The relative volume of the head decreases from about 27 per cent in the infant to 7 per cent in the adult. The relative surface-area decreases from about 18 per cent in the infant to 6.5 per cent in the adult. The ratio of relative surface-area to relative volume therefore changes from about 2:3 in the infant to nearly 1:1 in the adult. The relative surface-area and volume of the head are probably usually greater in short than in tall individuals, in men than in women, and in the thin than in the fat.

The relative volume of the lower extremities increases from about 15 per cent in the infant to about 30 per cent in the adult. The relative surface-area increases from about 25 per cent to about 40 per cent. The ratio of relative surface-area to relative volume is about 5:3 in the infant, 4:3 in the adult. Both relative volume and relative surface-area are greater in women than in men, in tall than in short individuals, and in the thin than in the fat.

The relative volume and surface-area of the upper extremities are remarkably constant throughout life. The volume is approximately 10 per cent of the total volume, the surface-area nearly 20 per cent. The ratio of relative area to relative volume is therefore nearly 2:1. Both relative volume and surface are less in women than in men, in the thin than in the fat.

A few examples will suffice to illustrate the relation of volume and surface-areas of the different segments of each limb to the limb as a whole:

TABLE 8.

No.	Stature.	Sex.	Index.	Lower extremity = 10.						Upper extremity = 10.					
				Surface.			Volume.			Surface. <sup>1</sup>			Volume.		
				Thigh.	Leg.	Foot.	Thigh.	Leg.	Foot.	Arm.	Forearm.	Hand.	Arm.	Forearm.	Hand.
1	50.0	Male.	0.0242	3.8	3.5	2.7	.....	.....	.....	4.3	3.0	2.7	.....	.....	.....
2	55.0	Male.	.0238	.....	.....	.....	5.5	3.0	1.5	.....	.....	.....	5.5	3.1	1.4
3	73.2	Fem.	.0160	4.6	3.3	2.1	.....	.....	.....	6.9	.....	3.1	.....	.....	.....
4	77.0	Male.	.0150	.....	.....	.....	4.6	3.6	1.8	.....	.....	.....	5.0	3.3	1.7
5	149.7	Fem.	.0277	5.2	3.2	1.6	.....	.....	.....	7.7	.....	2.3	.....	.....	.....
7	164.3	Male.	.0144	4.9	3.2	1.9	.....	.....	.....	7.2	.....	2.8	.....	.....	.....
9	164.8	Fem.	.0129	5.0	3.4	1.6	.....	.....	.....	7.3	.....	2.6	.....	.....	.....
10	156.0	Fem.	.0159	.....	.....	.....	6.8	2.3	0.9	.....	.....	.....	6.0	3.0	1.0
12	170.0	Male.	.0121	4.3	3.9	1.8	.....	.....	.....	3.8	3.4	2.8	.....	.....	.....
13	169.4	Male.	.0141	.....	.....	.....	6.1	2.9	1.0	.....	.....	.....	6.0	2.8	1.1
14	171.0	Male.	.0157	5.1	3.2	1.7	.....	.....	.....	3.9	3.4	2.7	.....	.....	.....
15	178.0	Male.	.0114	5.1	3.3	1.6	.....	.....	.....	7.2	.....	2.8	.....	.....	.....
16	179.2	Male.	.0130	5.0	3.3	1.7	.....	.....	.....	7.6	.....	2.4	.....	.....	.....

<sup>1</sup> For the Du Bois cases the surfaces of the arm and forearm are combined.

An inspection of this table will show that while the relative volume of the hand decreases from infancy to maturity, and that of the arm increases, the relative surface-area of the three segments remains remarkably constant. Relative to the limb as a whole, the volume and surface of forearm are nearly equivalent, while the surface of the hand is increased relatively to its volume and that of the arm decreased. In fat individuals and in very tall individuals the volume and surface of the hand are relatively small.

In the lower extremity the relative volume and surface-area of the foot are both decreased, those of the thigh are increased from infancy to maturity, while those of the leg are little altered. The proportional relations of volume to surface in the three segments are comparatively little changed. The volume of the female thigh is relatively large, but the surface does not appear to be so. It is interesting to observe that in the excessively fat woman, No. 5, the proportional area of the three segments of the lower extremity are nearly like those of other adults, while the hand is relatively small.

### LINEAR PROPORTIONS.

In the study of the proportions of the body it is of fundamental importance to have standards of typical proportions with which the proportions of a given individual or of a group of individuals of a given race and stage of development may be compared. The most extensive set of standards of this kind is that prepared by Quetelet (1870) in his study of Belgians. He gives tables of mean absolute and

relative measurements of height, weight, and of a large number of linear measurements of the body in groups of each sex at birth, at yearly intervals from 1 to 20 years, and at 25, 30, and 40 years of age. The standard group utilized consisted of 10 individuals, but he frequently makes use of larger groups as well as of groups chosen at other intervals than those above mentioned, and of especially selected individuals. We thus have a good set of Belgian standard types. Some of Quetelet's data are reproduced in table E. There would be an advantage in having means based on larger groups but, as Quetelet pointed out, the advantage is less than might be anticipated.

Weissenberg (1911) has furnished a similar set of standard type proportions of South Russian Jews based on similar age-groups but extending the groups to ages 50 and 60 years. He has tabulated a much less extensive number of linear proportions than Quetelet, but makes use of a larger number of individuals (50) in a given group from which to estimate the mean. Some of Weissenberg's data are tabulated in table D.

In America the best study of proportions by age-groups is that of Hastings (1902), whose observations extend from 5 to 20 years of age and are based on a large number of individuals for each age. His tables mark a step in advance over those above mentioned in that each age-group is subdivided into height-groups. The number of linear measurements given is, however, relatively limited. Some of these are utilized in table F. For the period of infancy and childhood up to 5 years and for maturity and old age we have but few American data. Extensive studies have, however, been made of the bodily proportions of college boys and girls, the most important of which are those of Hitchcock, Seaver, Sargent, and Barr. S. B. Moon (1892) and W. S. Hall (1896) have furnished valuable data on bodily proportions immediately preceding and during adolescence.

Godin (1903, 1910) has furnished even more extensive data on the development of bodily proportions in French youths 13.5 to 17.5 years of age. For younger children we have the data of Kotelmann (1879) on German school children, of Landsberger (1888) on school children in Posen, and of Ernst (1906) and of Schwerz (1910) on Swiss school children.

The studies of the investigators mentioned and of numerous others, on the whole, confirm the views advanced by Quetelet as to unity of type in bodily proportions. At a given stature and a given stage of physiological development the mean proportions of the human body are strikingly similar in diverse races. The similarities are so great that extensive careful statistical studies are necessary to prove conclusively that certain bodily proportions are characteristic of one race as opposed to another race. There is far more variation in rapidity of growth and in mean average adult stature in various races and social groups than there is in the proportions of the body relative to stature at a given stage of physiological development. For a discussion of racial differences in bodily proportions, so far as they at present appear to be determined, the reader is referred to Martin (1914). If we take into account stature, weight, and physiological development we may safely use statistical data from various sources in the endeavor to arrive at standard types of bodily proportions of Europeans and those of European descent.

In the study of the relative proportions of the body the most practical method is to express other linear measurements in terms of ratio to stature. In making stature the standard, however, it must be remembered that the stature is about 1 cm. greater in the recumbent than in the standing position; that after rest in bed at night the stature standing may be as much as 2.5 to 3 cm. greater than later in the day,<sup>1</sup> and that the length of a fresh cadaver is greater by about 2 cm. than the stature during life.

*Grouping by age and stature.*—The chief disadvantage, however, in the use of most of the data referred to above comes from the fact that as a rule the various measurements of the body have been studied primarily from the standpoint of age rather than from that of stature and have been averaged for a given age rather than for a given stature. Thus we may find the average stature of a given group of boys between the eighth and ninth birthday is 126.1 cm., the average sitting-height 68 cm. We may assume that 68 cm. approximates the average sitting-height for a stature of 126.1 cm. and that the average ratio of height to sitting-height for a child with a stature of 126.1 cm. is approximately 54 per cent. Since, however, children of a given age vary considerably in size the approximation is at best a somewhat rough one. To get accurate data concerning relative bodily proportions the children should be grouped primarily according to stature, not age—or if grouped according to age the age-group should be subdivided into stature-groups. Hastings (1902) has followed the latter method and has recorded his data in such a way that it is possible to compare proportions based on measurements recorded according to age with those recorded according to stature. Thus we find:

TABLE 9.

School boys.	Mean sitting-height, in centimeters.	Relative sitting-height, per cent stature.
Mean stature, age 9 (125.86 cm.)...	68.00	54.0
Group with 127 cm. stature, age 8...	69.82	55.0
Group with 125 cm. stature, age 8...	67.86	54.3
Group with 126 cm. stature, age 9...	68.67	54.5
Group with 126 cm. stature, age 10...	68.20	54.1

From these data it may be seen that in this case the relative sitting-height based on age-group statistics is lower than that based on stature-groups of similar height. Hasting's stature subgroups are, however, rather comprehensive groups. It is of interest to compare the data just given with data based on the records of the individual cases published by Boas and Wissler, 1904. For boys between 7.1 and 11.8 years of age there are records of 40 with a stature between 126 and 126.9 cm. The average height is  $126.4 \pm 0.237$ , the average sitting-height is  $67.74 \pm 2.14$ , the relative sitting-height is 53.6 per cent of stature, a figure more nearly corresponding with Hastings age-group data than with his stature-subgroup data. Boas and Wissler's 40 cases may be subdivided into 4 age-groups of 10 each with an average

<sup>1</sup> Curtiss (1898) followed the nightly loss in weight and gain in stature in three young men daily for a school year. The average loss in weight was 0.79 pounds, the average gain in stature about 18.22 mm.

age respectively of 7.92, 8.96, 9.87, and 10.9 years. The relative sitting-height of these groups is respectively 53.5 per cent, 53.4 per cent, 53.2 per cent, and 54.3 per cent. The nearest general age-group stature given by Boas and Wissler is that of  $127.8 \pm 5.5$  cm. for the age of 9. For this age the sitting-height is given as  $68.5 \pm 3.0$ . The relative sitting-height is therefore 53.6 per cent of the stature, the same as that found as the average for the whole group of boys of 126 to 126.9 cm. stature.

For head-girth Hastings gives 52.49 cm. as the mean for the 9-year group (stature 125.86 cm.). The relative head-girth is therefore 41.7 per cent. For the 125 cm. group, age 8, the relative head-girth is 42 per cent; for the 127 cm. group, age 8, it is 41.8 per cent; for the 126 cm. group, age 9, it is 41.4 per cent; for the 126 cm. group, age 10, it is 41.3 per cent. Ernst (1906) gives individual data on head-girths of Swiss school children, 8 to 15 years of age, which make possible a comparison between groups based on stature and on age. For age 8 to 9 Ernst found the mean stature of boys to be 126.1 cm., the mean head-girth 52.0 cm., the relative head-girth 41.3 per cent. For age 9 to 10 the mean stature was likewise 126.1 cm., the mean head-girth 52.0 cm., and the relative head-girth 41.3 cm. Between the ages of 8 and 11 she gives data on 10 boys with a stature between 126.0 and 126.8 cm. (average 126.35). The average relative head-girth for this group is 41.06.

When stature-groups are used the variability of the different measurements is much less than when age-groups are used. Ernst has given data which make it possible to compare the mean variability of certain measurements according to stature and age-groups. The following data illustrate the greater variability in the age-groups:

TABLE 10.

Age, years.	Sex.	Stature.		Mean weight.	Per cent of average deviation of—			No. of cases.
		Range.	Mean.		Weight.	Chest-girth.	Head-girth.	
		<i>centimeters.</i>	<i>cm.</i>	<i>kilos.</i>				
8 to 9	Male.	115.7 to 133.4	126.1	24.7	6.1	3.2	14.3	25
9 to 10	Male.	116.3 to 135.6	126.1	25.3	8.5	2.6	18.8	25
8 to 13	Male.	126 to 126.9	126.3	26.4	4.1	3.3	2.2	10
11 to 12	Fem.	126.1 to 145.7	137.1	31.3	8.9	3.2	14.7	25
10 to 15	Fem.	137 to 138.9	137.8	30.6	6.8	3.0	2.06	11

The data of Boas and Wissler (1904) likewise make it possible to compare the variability of measurements in age-groups with those in stature-groups:

TABLE 11.

Age, years.	Sex.	Stature.		$\sigma$	Mean weight.	$\sigma$	Height, sitting.	$\sigma$	Width of head.	$\sigma$	Length of head.	$\sigma$	No. of cases.
		Range.	Mean.										
		<i>cm.</i>	<i>cm.</i>		<i>lbs.</i>		<i>cm.</i>		<i>cm.</i>		<i>cm.</i>		
9.....	Male.	127.8	127.8	5.5	59.4	7.3	68.5	3.0	14.40	0.47	18.12	0.63	220
7.1 to 11.3	Male.	126 to 126.9	126.4	0.2	58.3	4.7	67.7	2.1	14.33	0.40	18.05	0.55	40
11.....	Fem.	137.2	137.2	6.4	70.0	10.4	72.6	3.6	14.21	0.49	17.82	0.54	199
8.4 to 13.5	Fem.	137 to 138.9	137.84	0.6	70.0	6.46	72.6	2.2	14.31	0.42	17.97	0.64	40

This table shows greater variability in the age-groups than in the stature-group in all measurements except that of width of head in girls.

*Curves of linear proportions.*—In charts I and J, pages 534, 535, we have plotted curves to illustrate the alterations in the relative proportions of various linear measurements to stature which take place during growth in individuals of normal build. Curves of the height-weight index of growth are inserted to illustrate the course of growth of individuals who reach average American stature (63 inches for women, 67.5 to 68 inches for men). A curve ( $x$ ) is also inserted to illustrate changes in “average transverse diameter” based on the square root of a tenth of the height-weight index of build. Where curves for females depart markedly from those for males the former are indicated by broken lines. The ages given at the bottom on chart I and at the right of chart J represent the approximate age at which an average healthy American reaches the stature shown at the opposite side of the chart. Considerable variation in rapidity of growth may exist without producing marked alterations in the growth-curves shown in the chart. Figures on which these curves are based are given in table K, page 549.

The curves may be considered as to periods of infancy, childhood, adolescence, and maturity.

During infancy rapid growth in length may alternate with rapid growth in weight (thickness), but available data seem to show that up to the period of the first dentition the two processes essentially keep pace. We have not many statistics relating to variation in the proportions of the body during early infancy. Weissenberg (1911) found no essential variations up to the third month after birth. The figures given in table A, based on data from Schmid-Monnard, show no uniform change in relative chest-girth until the latter half of the first year, and an examination of the available data relating to the height-weight ratio has led me to similar conclusions. In the absence, therefore, of more definite data I have plotted the curves of linear proportions for infancy on the assumption that there is normally little alteration in relative proportion of the body during this period. There is undoubtedly considerable variation in speed of development.

During childhood, as we have seen, the proportions of the body are primarily correlated with stature rather than with age. The children of stature 126 to 126.9 cm. and 137 to 138.9 cm., in table 10, show no definite correlation between age and weight or chest-girth. The head-girth appears to be slightly smaller in the older than in the younger children. The older boys of stature 126 to 126.9 cm., in table 11, show a slightly smaller average weight than the younger (57.2 pounds at 11 years as compared with 58.8 pounds at 8 years) and a greater sitting-height (68.6 cm. at 11 years as compared with 67.6 cm. at 8 years). The diameters of the head in the 11-year-old group are slightly smaller than in the younger groups. In girls of stature 137 to 138.9 cm. the sitting-height increases progressively from the 10-year-old to the 13-year-old group (from 72 to 73 cm.). The weight is greater in the 13-year-old group than in the younger groups (74 pounds as compared with 68.6 pounds). No correlation between diameters of the head and age is apparent.

Variations in bodily proportions, due to acceleration or retardation of the usual course of development during childhood, affect breadths and girths rather than

lengths. At certain periods children of a given stature who are retarded in growth average a greater body-weight than children not so retarded. Such children have some girths relatively increased. This subject is taken up in the following section on the relation of the height-weight index of growth to bodily proportions.

On the transition from childhood to adolescence, and during adolescence, chronological age, so far as it is correlated with physiological age, has a marked influence on relative bodily proportions. In vertical relative measurements the most marked change is an increase in length of trunk and decrease in length of lower extremities. These alterations, by no means uniform, are illustrated by the following table based on data from Hastings:

TABLE 12.

Stature 142 cm. (55.9 inches) Sitting-height.			Stature 160 cm. (63 inches) Sitting-height.			Stature 164 cm. (64.57 inches) Sitting-height.			Stature 172 cm. (67.7 inches) Sitting-height.	
Age.	Male.	Fem.	Age.	Male.	Fem.	Age.	Male.	Fem.	Age.	Male.
	<i>cm.</i>	<i>cm.</i>		<i>cm.</i>	<i>cm.</i>		<i>cm.</i>	<i>cm.</i>		<i>cm.</i>
11	75.43	75.50	14	83.00	84.47*	14	87.07	86.25	.....	.....
12	75.49	74.52*	15	84.16*	85.05	15	85.29*	.....	16	90.64*
13	74.86	75.15*	16	84.58*	85.25	16	85.72	87.75	17	90.50
14	75.50*	.....	17	84.44	85.79*	17	86.51*	88.04*	18	90.43*
.....	.....	.....	18	84.50*	84.06	18	86.63*	84.88	19	.....
.....	.....	.....	19	.....	84.13	19	88.00	85.50	20	91.17
.....	.....	.....	20	.....	85.05	20	88.15*	84.80	.....	.....

\* Estimates from data on groups of neighboring statures.

Relative breadths and girths are increased during adolescence. This increase in some measurements ceases when full stature is reached, in others it may continue up to 50 years of age or longer.

The changes in relative proportions during adolescence and early adult life cause elevations and dips in some of the curves in charts I and J. The position of these elevations and dips depend on the average stature reached by the group of individuals studied. As shown in chart I, they are centered about an average adult stature of 63 inches for women, 67.5 to 68 inches for men. If a group of adults of shorter average height is studied, the elevations and dips shift to the left in each curve; if a group of greater adult height is studied, they shift to the right and as a rule are less marked. The older the group the more marked the dips. In charts I and J the dips are those characteristic of young adults.

In plotting the curves in charts I and J we have endeavored to illustrate the broader features of typical changes in bodily proportions. In the course of the growth of any given individual or group of individuals minor fluctuations occur which are not indicated in the charts. Periods of relatively rapid growth in length are described as alternating with periods of relatively rapid growth in bulk. In the growth of the limbs rapid increase in length is usually accompanied by a decrease in relative girth. Godin (1903) has described alternate periods of increase in relative length and in relative girth of the long bones of the limbs; he states that these periods are reciprocal for the two osseous segments of a given extremity. Moon (1892) found in boys, aged 11 to 15, rapid increase in length of arm and leg preceding

rapid growth in length of thigh and forearm, but he found acceleration in growth of bone-girths accompanying growth in length of the long bones of the limbs, acceleration in muscle-girths following this period.

Having thus reviewed some of the fundamental features of relative linear proportions we shall take up briefly a more detailed consideration of certain of the more important of these measurements.

#### VERTICAL MEASUREMENTS.

(Chart I, p. 534; Table K, p. 550.)

These measurements are taken from the horizontal level of one point to that of another, not directly between the points named.

*Vertex to external acoustic meatus.*—The curve of the external meatus follows, with a few slight modifications, the data of Quetelet (see table E). For the taller individuals I have had the data of Mr. Puestow, referred to in connection with the discussion of head-volume, page 493. The data of Hrdlička (1899) and of Godin (1910) correspond closely with those of Quetelet. The Swiss children reported by Ernst (1906) and Schwerz (1910) give smaller values by about 0.5 per cent of the stature. Girls give slightly smaller values than boys of the same stature. It is the relatively slow growth of the part of head above the external acoustic meatus that chiefly accounts for the decrease in the relative height of the head from infancy to maturity. Correlated with this relatively slow growth of the cranial portion of the skull we find, according to the data of Mies (1894), that the relative weight of the brain decreases from about 17 per cent of the weight of the body in the infant to 2.86 per cent at 18 to 19 years of age. In adults of average build it appears to be in the neighborhood of 2.5 per cent.

*Vertex to top of larynx.*—I have chosen the level of the top of the larynx to mark the boundary between the head and the trunk because of its convenience for use in the study of head-volume and because the structures above this level, including the upper cervical region, all belong essentially to the head. In relation to the vertebræ, however, this level shifts downward during development. According to Symington (1887), in the infant at 1 year the upper border of the thyroid cartilage lies opposite the junction of third and fourth cervical vertebræ; in a 6-year-old child, opposite the body of the fourth cervical vertebra; in the adult, opposite the upper margin of the body of the fifth cervical vertebra. In the living it lies somewhat more distal than in the cadaver position above described, and in the aged may sink considerably lower. The curve given is based on measurements I have made in a series of children and young adults. It runs fairly close to a curve based on Quetelet's data on vertex to the "*fin du menton*." (See table E.) Zeising (1858) apparently selected a lower point on the larynx than that selected by me. The data of Godin (1910) correspond more nearly with Quetelet's *vertex, naissance du menton* than with his *vertex, fin du menton* data, and give lower values by about 1 per cent of the stature. Landsberger (1888) gives data which closely correspond with those here plotted for children. He measured from chin to vertex.

While there are well-marked individual variations, the relative height of the head usually varies with stature and is less in tall than in short individuals. Accord-



ing to the general tables of Quetelet, in girls during adolescence and in women it is less than in males by about 0.5 per cent of the stature. The relative height of head which Quetelet gives for selected female models is, however, equal to that of males of the same stature.

The difference between the level of the top of the larynx and that of the external acoustic meatus may be used as a measure of the facial portion of the head. Relative to stature, this distance is slightly increased at the time of the transition from infancy to childhood and thereafter slowly decreases until adolescence. According to Godin's data there is a very slight relative increase in the distance external acoustic meatus to chin between adolescence and maturity.

*Height of acromion.*—This curve follows closely the data of Weissenberg (see table D). Quetelet (1870) gives data on the distance from the vertex to clavicles. This distance is from 1 to 2 per cent of the stature shorter than that from the vertex to the sternal notch. Landsberger's data (1888) correspond closely with my curve. Godin (1910) finds the level of the acromion about 1 per cent of the stature lower than that shown by my curve during adolescence but at about the same level in the adult. He gives the level of the sternal notch during adolescence at about the height of my curve for the acromion. The acromion in youths of European descent appears to be generally about 1 per cent of the stature lower than the sternal notch during adolescence, but less than this in the adult. Martin (1914) gives the distance between the two levels as about 8 to 10 mm. in Europeans. In my curve I have represented the curve of the level of the acromion at a distance of 18.5 per cent of the stature from the vertex at a height of 67 inches and above. This corresponds with the relatively few observations I have made on young adults. Hitchcock's data on college students (table L) show the distance from vertex to sternum to be greater in short than in tall individuals. The difference is slight, however, compared with the difference in the relative height of the head. Tall individuals usually have long necks. I have found in the data studied no definite sexual differences. According to Weissenberg's data the relative distance vertex to acromion is only 24.4 per cent of the stature in the new-born. Quetelet (1870) gives the distance vertex to clavicle as 28 per cent. The intermediate value of 26 per cent has been chosen here. Weissenberg's data show a slight decrease in old age in the distance vertex to acromion.

Godin takes the distance external acoustic meatus to sternal notch as neck-length and states that this distance remains practically constant at one-tenth of the stature from 13.5 to 17.5 years of age. From 17.5 to 23.5 years of age he finds a gain of 0.3 per cent of the stature.

*Sitting-height.*—This distance represents very nearly the difference between the stature and the free part of the lower extremities, the distance from the sole to the crotch. For the sake of simplicity I have assumed that stature *less* sitting-height represents the latter distance and the corresponding curve is based on data obtained in this way. Direct measurement of the distance sole to crotch is difficult and somewhat uncertain owing to the nature of the soft parts in the latter region. In infants, however, sitting-height has to be measured indirectly by subtracting distance sole to crotch from length. In adults, according to Bertillon (1889), the distance sole

to crotch is somewhat greater than distance stature less sitting-height. The difference is more in short than in tall men. A few examples may illustrate this point:

TABLE 13.

Stature.	Stature less height sitting— Stature = 100.	Sole to crotch— Stature = 100.	Difference.
143 to 147 cm. .	44.6	47.4	2.8
163 to 167 cm. .	46.8	48.5	1.7
178 to 182 cm. .	48.1	49.2	1.1

This difference may possibly be due either to the relatively greater adiposity of the buttocks in short individuals or to a greater inclination of the pelvis.

It appears probable that the level labeled "crotch" in chart 1 may be from 1 to 2 per cent lower than the distance that would be obtained by direct measurement. Quetelet (table E) gives data for the distance sole to "bifurcation," which give a curve essentially similar to but in general slightly lower than mine. It shows, however, no decrease in relative distance during adolescence and is slightly higher than mine for the adult.

My curve, as stated above, is based on a study of data on sitting-height. The most extensive series of such data are those of Weissenberg, table D, which extend from infancy to old age. Valuable data on American school children are given by Peckham (1881), Porter (1893), West (1893, 1894), Hastings (table F), Smedley (1900), Boas and Wissler (1904), and other investigators. Godin (1910) has given data on French youth. Curves based on data from these various sources, while they show some minor variations, correspond essentially with the curve given in chart I. All show a decrease in relative sitting-height from infancy to the time of puberty and an increase after puberty. According to Weissenberg's data, table D, the period of relatively least sitting-height or greatest length of free lower extremity is at the age of 13 for girls, 15 for boys. After puberty the relative sitting-height increases rapidly until the age of 18, then slowly to the age of 21 to 25, remains nearly constant until the age of 41 to 50, and then increases. In old age it again decreases. In females it attains a maximum at the seventeenth year, then with slight variations it remains nearly constant until old age, when it again declines. In the adult the relative sitting-height of women is about the same as that of men of the same stature; according to Manouvrier (1902) it is less. Quetelet (table E) shows the distance sole to crotch to be slightly less in the female than in the male after infancy.

The distance "sitting-height" less the distance "vertex to acromion" makes a convenient measurement for the trunk. It will be noted in chart I that this distance decreases from about 41 per cent of the stature in the infant to 33.3 per cent at the period preceding puberty and then increases to maturity, except in unusually tall individuals. If we divide the neck between the head and the trunk we may measure the vertical height from the top of the larynx to the seat. Thus measured, the trunk shows a similar decrease in relative length from infancy to late childhood and a

subsequent increase during adolescence. (See table K.) For data on the relative length of the trunk measured from the sternal notch to the symphysis pubis during growth the reader may consult Godin (1903, 1910) and Schwerz (1910); for the length of trunk measured from the seventh cervical vertebra to the end of the sacrum, O. Ranke (1905), Ernst (1906); for the distance first palpable vertebra to the end of the sacrum, Quetelet (1870).

*Height of iliac crest.*—There are few available data on the relative height of the iliac crest during growth. The curve shown in chart I is based on comparatively few observations and can be looked upon merely as a tentative curve. So far as I am aware, Zeising (1858) is the only author who has endeavored to give figures of the height of the top of the crest during the whole growth-period. The curve in the main follows his data but has been checked up by a few observations of my own. It represents the highest point of the crest. The anterior superior iliac spine, which has been much more frequently measured, is about two-fifths of the distance between this point and the ischial tuberosity. Landsberger (1888) appears to have measured to a point on the crest ventral to and lower than the top of the crest.

*Height of anterior superior iliac spine.*—The curve for the anterior iliac spine has not been plotted in chart I because the somewhat limited data at hand do not give concordant results. The level which Quetelet designates “aux hanches” appears to be somewhat higher than the anterior superior iliac spine, but lower than the higher part of the iliac crest. The data of Schwerz (1910) cover merely the school-child period. For the younger children the data of Schwerz show the iliac spine about 2 per cent of the stature below the corresponding figures of Quetelet. For the older individuals the difference is about 1 per cent of the stature. Schwerz's data show the iliac spine at 53.9 per cent of the stature in boys 45 inches tall, 57.7 per cent in boys 62 inches tall, and 57.3 per cent in boys 66.7 inches tall. The curve for girls of similar height corresponds closely with that for boys. Godin (1903) gives slightly lower values. According to this author the relative increase in the height of the iliac spine which takes place about the fifteenth year is in part due to the decrease in the inclination of the pelvis which takes place at this period.

*Top of pubic crest.*—No curve has been plotted for this height in chart I because of the confusion such a curve might make with that plotted for the top of the trochanter. Quetelet (table E) gives data for this level from birth to maturity. A curve based on his data runs nearly parallel with and about 12 per cent of the stature below the curve for the iliac crest shown in chart I. Hall (1896) gives data on the height of the pubic crest in American boys 50 to 68 inches in height, which closely parallel Quetelet's data. Godin (1910) gives a relatively greater height by about 2 per cent of the stature for French boys 13.5 to 17.5 years of age. The data given in table L on American college students correspond in value with those given by Quetelet for adults, but are slightly lower for the men (50.3 as compared with 50.8). The American college statistics show the crest relatively slightly lower in short than in those of medium height and tall individuals.

*Top of the great trochanter.*—The data of Weissenberg (table D), Godin (1910), and Ernst (1906) correspond closely with the curve for this level given in chart I

and in table K. Quetelet's data (table E) give values from 1 to 2 per cent of the stature lower.

*Length of lower extremity.*—Compared with the length measured from the trochanter, the difference in the length of the lower extremity measured from various other levels amounts approximately to the following percentages of the stature:

From crest, 8 to 10 per cent of stature, greater.

From iliac spine, 4 to 5 per cent of stature, greater. (Greater after puberty than before. Greater in girls than in boys.)

From pubis, 1 to 2 per cent of stature less. (Greater before than after puberty.)

From stature minus sitting-height 4 to 5 per cent of stature less. (Greater before than after puberty.)

From sole to crotch 3 to 4 per cent of the stature less.

According to the data of Weissenberg (table D) the difference in relative length of the lower extremities measured from the trochanter and measured by subtracting sitting-height from stature is greatest in the infant, decreases rapidly during the first three years after birth, and then more slowly until the tenth to twelfth year. In males it now once more begins to increase and this increase continues at first rapidly, then slowly to the fiftieth year. At 15 in males, during a period of rapid growth in length, it does, however, show a temporary decrease. In late adolescence it increases. In old age it decreases. In females it increases to the seventeenth year and then remains nearly constant until old age, when it decreases.

The decrease in difference in the relative length of the two measurements of the lower extremities following infancy is due mainly to change in shape of the infantile femur and pelvis and in the form of the lumbo-sacral curve. There is during this period a relative increase in the length of the lumbar region of the spinal column (Bardeen 1905). The increase during adolescence and in the adult male is probably to be ascribed partly to skeletal changes, partly to the accumulation of fat on the buttocks. To this cause may also, in part at least, be ascribed the differences between males and females, the female in youth and maturity showing as a rule a greater difference between the two measurements under consideration than the male. Weissenberg's data show that the relative length of the lower extremities measured from the trochanter is about the same in boys and girls of the same stature up to 48 inches height (9 years of age), but that after the tenth year the lower extremities grow faster in relative length in boys than in girls, reaching a maximum of 53.2 per cent of the stature at the fifteenth year (height 60.5 inches). In girls the maximum relative length of the lower extremities (52 per cent of the stature) is reached at the thirteenth or fourteenth year (height 57 to 59 inches). After the period of maximum relative length in both sexes there is a decline in relative length of the lower extremities amounting to about 1 per cent of the stature.

*Height of knee-joint.*—Data on the height of the knee-joint are unsatisfactory. Technical difficulties in locating the level of the joint cavity have led to considerable variation in landmarks chosen and in results reported. Quetelet (table E) gives the relative height of the patella as progressively increasing from 23 per cent of the stature in infancy to 28.4 per cent in the adult male, 28 per cent in the adult female. He fails to note here, as in many of his data on growth, the influence of puberty on the growth-curve. In general his data give a somewhat higher curve for males than that shown in chart I but about the same curve for females. The data on the

French boys given by Godin (1910) correspond fairly well with my curve. Landsberger (1888) gives a greater relative height for the knee-joint than Quetelet. The Schwerz data (1910) show an average lower height of the knee-joint of nearly 1.5 per cent of the stature than that shown in my curve. Schwerz finds the knee-joint in girls relatively higher than that of boys of the same stature except at ages 9, 10, and 11, at which they are the same. The children studied were from 6 to 14 years of age. There is a conflict in recorded data as to the relative height of the knee in males and females. The limited number of observations I have made show the female knee-joint slightly higher in adolescents. The relative heights of the knee-joint given in table L are not comparable for men and women because of uncertainty of similarity of technique of measurement. Hitchcock (table L) shows the knee-joint lower in short than in tall individuals and higher in young than in mature men.

*Height of ankle-joint.*—The top of the joint cavity lies opposite the prominence of the medial malleolus, not its tip. The curve labeled "ankle-joint" in chart I shows the former level and hence is somewhat higher than it would be if it represented the latter level. The data given by Quetelet (table E) for males correspond with the curve shown in chart I. Quetelet gives a lower height for the female than the male ankle-joint, which equals 0.5 per cent of the stature in the adult. Godin (1910) gives a uniform height of 4.5 per cent of the stature during adolescence, 4.6 per cent in the adult. Schwerz (1910) gives a height of 4.3 per cent of the stature at the age of 6, 4.2 at the age of 14 for boys, 4.7 per cent and 4.2 per cent at the same ages for girls. Weissenberg (1911) gives the relative height of the foot in male infants as 6.5 per cent, of female infants 6.1 per cent. In the adult the figures are respectively 4.7 per cent and 5.0 per cent.

*Segmental proportions of lower extremity.*—Comparing the parts of the lower extremity in infant and adult, we obtain the following approximate proportions of height of segment to length of limb:

TABLE 14.

	Free limb = 100.			Limb from trochanter = 100.			Limb from crest = 100.		
	Infant.	Adult.		Infant.	Adult.		Infant.	Adult.	
		Male.	Fem.		Male.	Fem.		Male.	Fem.
Thigh.....	33.3	43.2	41.5	45.7	48.3	46.3	56.0	56.2	56.1
Leg.....	48.5	46.7	48.3	39.5	42.5	44.3	32.0	35.9	36.3
Foot.....	18.2	10.1	10.2	14.8	9.2	9.4	12.0	7.9	7.6
Leg and foot...	66.7	56.8	58.5	54.3	51.7	53.7	44.0	43.8	43.9

From these figures it may be seen that the thigh changes little in length relative to the limb as a whole measured from the trochanter or from the iliac crest. On the other hand, from the standpoint of the free extremity the leg changes comparatively little in relative length while the thigh elongates. In all cases the relative height of the foot is reduced.

*Length of foot.*—This measurement has not been plotted in chart I, but it is tabulated in table K. Weissenberg's data (1911) show an increase in relative

length of foot from 15.3 per cent of the stature at birth to 16.4 per cent at 9 years, little change from here to puberty, and then a decline to 15.7 per cent at 20 years of age. Quetelet (1870) gives the relative length of the foot at birth in each sex as 15 per cent of the stature. In the adult he gives the length of the male foot as 16 per cent, the female foot 15 per cent. In both sexes he shows the foot longest at about the period of puberty. This is likewise shown by the data of W. S. Hall (1896) on American boys. Hall gives a relative length of the foot that is about 0.5 per cent of the stature less than Quetelet's for corresponding stature, but Hall's measurements appear to have been taken with the knee flexed and the foot on a platform relieved of the body-weight. The length of foot of American college students is likewise usually taken in this way (Seaver, 1909). The length of the foot relieved of the body-weight is usually shorter than when bearing body-weight. The data of Bertillon (1889) on length of foot in relation to stature in men show that short men have relatively longer feet than tall men. At a stature of 57 inches the length is 16.1 per cent of the stature, at 73 inches it is 15.5 per cent. The female foot is relatively shorter than the male foot. The difference begins in childhood and in the adult amounts to about 0.5 to 1 per cent of the stature.

*Length of upper extremity.*—This is plotted for males in chart I between the lines "height to acromion" and "height to tip of middle finger." The data for the curves are given in table K. The total length increases from 41.5 per cent of the stature at birth to 45 per cent in the adult. The female extremity is about 1.0 per cent of the stature shorter than the male. Its length is not plotted in chart I. Data on the growth of the upper extremity are given by Weissenberg (1911), Quetelet (table E), Godin (1910), Schwerz (1910), Ernst (1906), Landsberger (1888), and others. The differences in relative length at a given stature found by these various investigators are slight and will not be discussed here. The length given for the upper extremities by Schwerz (1910) is 0.5 to 1 per cent of the stature less than that shown in my curve. The difference comes chiefly in forearm and hands. The data of the other investigators come closer to my curve. Weissenberg (1911) states that the upper extremity reaches its full length in females at 18 years of age, in males at 25 years of age. Pfitzner (1899) reports growth continuing until old age.

*Length of arm.*—For level of acromion to level of elbow-joint, see chart I. The length of the arm given by Quetelet (1870) and by Landsberger (1888) is about 1 per cent greater than that shown in my curve. This is probably due to differences in the technique of measurement used. Hall (1896), who measured to the olecranon, gives still higher figures for the relative length of the arm. The data of Schwerz (1910) correspond closely to my curve, but cover a period merely from the sixth to the twentieth year. Sexual differences in relative length of the arm are not apparent in the data of Schwerz. Quetelet makes the female arm slightly shorter than that of the male. Hitchcock (1900) shows that the right arm averages uniformly longer than the left. According to Godin (1910), the arm changes little in relative length from 13.5 to 17.5 years of age, but at 23.5 years of age is 0.7 per cent of the stature greater than that at 13.5 years of age.

*Length of forearm.*—This is shown in chart I by the distance between the curve for the elbow-joint and that for the wrist-joint. The former represents the top of the radius, the latter the lower joint surface of the radius, not the tip of the styloid process. The data of Quetelet (table E) show the distance from elbow-joint to wrist-joint less than that shown in chart I, but the distance acromion to wrist-joint is closely similar until maturity is approached, when it becomes slightly longer than that shown in chart I. Godin (1910) gives lower values for length of forearm in the younger boys of his series, about the same values for the older boys. The data of Schwerz (1910) correspond closely with the curves in chart I for length of forearm, but since he measured to the tip of the styloid process the length of the forearm is relatively less. Both Quetelet and Schwerz show the female forearm shorter than the male. The difference amounts to about 0.5 per cent of the stature. It is evident after the fifth or sixth year. According to Godin (1910), the forearm attains its greatest relative length at 16.5 years of age. It is 0.4 per cent of the stature longer at 23.5 years of age than at 13.5 years of age.

*Length of hand.*—This is measured from the wrist joint to the tip of the middle finger, “medius.” Data on the relative length of the hand from birth to maturity are given by Quetelet (table E) and by Weissenberg (1911). Daffner (1902) gives a detailed comparison of the hand and feet of the new-born and adults. Godin (1910) and Schwerz (1910) give data on the length of hand in school children. In table K data are given for the curves in chart I. The relative length of the hand there given for the new-born (12 per cent of the stature) is somewhat less than that given by Weissenberg (males 12.6 per cent) and Quetelet (12.2 per cent for both sexes). In the cases I have studied I have found the length slightly less than 12 per cent and this is true of the few cases tabulated by Meeh (table B). For older children, adolescents, and adults the relative hand-length shown in chart I corresponds closely with Quetelet’s data. It is somewhat greater than that of the younger children recorded by Schwerz and that of the Godin adolescents. In part this is due to basing the curve on the wrist-joint rather than on the tip of the styloid process. The hand decreases slightly in relative length from birth to maturity. There appear to be no well-marked sexual differences in length of hand relative to stature. Relative to width of hand, the female hand is narrower (Daffner, 1902).

*Segmental proportions.*—The relative lengths of the various segments of the upper extremity to the extremity as a whole in the infant and adult are shown approximately in the accompanying table.

TABLE 15.

	Infant.	Adult.	
		Male.	Fem.
Hand.....	28.9	24.9	25.3
Forearm.....	31.3	32.9	32.8
Arm.....	39.8	42.2	41.9
Upper extremity..	100.0	100.0	100.0

## BREADTHS AND DEPTHS.

In chart J, p. 535, a few relative breadths and girths have been plotted. The data on which these curves are based are shown in table K. The midline of the chart represents zero. The distance to the right or left of this line represents the percentage of stature of a measurement of one-half of the body. No attempt is made to illustrate lateral asymmetry. Since relative adiposity influences transverse diameters and girths more than it does vertical measurements, we find greater variability in most of the measurements plotted here than in those of chart I, and therefore greater difficulty in plotting satisfactory curves.

*Span.*—Measurement of span is especially variable because of the difficulty of uniform posture for this measurement. At birth the span is less than the stature, but authors differ as to the period in childhood when span equals or exceeds stature. Weissenberg (table D) makes this period relatively early in childhood and relatively earlier in boys than in girls. According to his data it reaches a maximum of 104.1 per cent of the stature in boys at age 17, in girls 103.4 per cent of the stature at age 16. Hastings (table F) finds the span less than the stature up to the age of 11 in boys, 15 in girls. He finds its greatest relative length in late adolescence in both sexes. He finds it greater in tall than in short individuals. This is not evident in American college statistics (table L). It is greater in males than in females.

*Breadth of head.*—Quetelet (table E) gives data on relative breadth of the head which show that it decreases from 20 per cent of the stature at birth to 9.1 per cent in males and 9.3 per cent in females at maturity. Data for more limited periods of development are given by Landsberger (1888), Hrdlička (1899), Hastings (1902), West (1893), Boas and Wissler (1904), Ernst (1906), Schwerz (1910), and others. There is comparatively little difference in growth-curves based on data from these various sources. The head relative to stature is narrower in tall than in short adults (table L). The head relative to stature in older girls and in women appears to be slightly broader than in males. Most of the authors mentioned also give data on the anterior-posterior diameter of the head. Pfitzner (1899) states that the cephalic index is constant from birth to old age, Boas (1904) that it decreases slightly during growth. Hrdlička (1899) found the younger children he studied had rounder heads than the older children. The only evidence of such a condition found by Ernst (1906) was a slightly greater proportion of hyperbrachycephalics among the younger children she studied. According to Boas (1912), the female head is slightly more round than that of the male.

*Breadth of neck.*—Quetelet (table E) gives data for the male which correspond fairly well with the curve in chart J, except for infants. The relative width he gives for infants is 9.9 per cent of the stature. Zeising (1858) gives 13.6 per cent. In the infants I have measured I have found the average relative width 11 per cent. Quetelet's data show the female neck relatively wider than the male. I find no evidence for this in normal individuals. The statistics on college students (table L) show the female neck smaller than the male. Godin (1910) shows a marked increase in the relative width of the neck from 17.5 to 23.5 years of age.



*Bi-acromial breadth.*—This measurement, labeled “width shoulders” in chart J, should be made between lines drawn on the skin perpendicularly above the ends of the acromial processes. Usually it is made after pressing down the skin over the ends of these processes and there is thus introduced a highly variable factor which increases the diameter from 1 per cent upwards. Weissenberg (table D) and Quetelet (table E) give data on this measurement from birth to maturity. Data for more limited periods are given by Landsberger (1888), Hall (1896), and Godin (1910). The curves in chart J follow closely Weissenberg’s data except for infancy. The relative bi-acromial width for the new-born appears to be nearer 24 per cent of the stature than the 21 per cent given by Weissenberg. Quetelet gives 24.5 per cent and shows a decline in relative width from infancy instead of the increase in width in early childhood preceding the decline, described by Weissenberg. Quetelet’s data in general show somewhat higher values for the relative bi-acromial width than those of Weissenberg, as also do those of Godin, Hall, and Landsberger, and American college statistics. In part at least, as mentioned above, this greater relative width may be ascribed to technique of measurement. Weissenberg’s data show no clearly defined sexual differences. Quetelet gives smaller relative bi-acromial widths for the older girls and adult women than for men of the same stature. Godin’s data show a difference of 3 per cent of the stature between the bi-acromial and bi-humeral width of the shoulders in the adult and 2.2 per cent at 13.5 years. American college statistics of width of shoulders measured over the deltoid muscles, table L, show this width in the men to be about 3 per cent of the stature greater than in women, greater in short than in tall individuals and in mature than in young individuals of full stature.

*Breadth of chest.*—The curve given in chart J and the associated data in table K are based primarily on the data of Hastings (table F). The data of Godin and of Ernst correspond fairly closely. This width is considerably increased after full stature is approximately attained. Godin (1910) shows an increase from 15.7 per cent of the stature to 16.3 per cent from the eighteenth to the twenty-fourth year. Quetelet’s biacillary diameter (table E) gives somewhat larger values except in infancy. Zeising’s data (1858) for width of chest in infancy, 21.6 per cent of the stature, correspond with such observations as I have made. Sexual differences are uncertain. Hastings in general gives a greater relative width of chest for boys, Ernst for girls. The latter are exceptionally wide-chested.

*Depth of chest.*—Hastings (table F) gives data which show a depth of chest slightly smaller than that shown by similar data of Quetelet (1870), Hall (1896), and Ernst (1906). Ernst states that her data confirm those of Sack and Hrdlička in showing a more rapid growth in the transverse than in the antero-posterior diameter of the chest during childhood. She also finds boys relatively thicker-chested than girls. Godin (1903) shows a slight increase in the thoracic index between 13.5 and 17.5 years of age in boys.

*Depth of abdomen.*—This measurement is one of the most important from the standpoint of relative build since it gives us the best index of relative adiposity in individuals with otherwise normal abdominal structure. Unfortunately there are

few published data on the subject. Hall (1896) shows a decline in this relative measurement from 10.5 per cent of the stature at 9 years of age to 10.1 per cent at 16 years of age and a subsequent increase to 11.4 per cent at 23 years of age.

*Bi-crestal breadth.*—This measurement, labeled “width of hips” in chart J, represents the widest distance between the iliac crests plus the overlying tissues. Weissenberg (table D) has furnished data on this measurement from birth to maturity. The relative width he gives for infants (15.4 per cent) appears to be small and is smaller than that illustrated by Godin (1910). The relative width of the hips may, however, increase slightly from infancy to early childhood. It then declines to the period preceding puberty and subsequently increases so as to reach or nearly reach the proportions of early childhood. The data of Godin (1910) correspond well with those of Weissenberg, which my curve follows. The bi-crestal width is relatively greater in females than in males after early childhood.

The distance between the anterior superior iliac spines was 85 per cent of the bi-crestal width in the boys 13.5 years of age studied by Godin (1903); 88 per cent at 16.5, 87 per cent at 17.5. In infancy and early childhood the differences in the two widths appear to be less. Quetelet (table E) gives data on the bi-ilio-spinal index from birth to maturity. Godin (1903) and Landsberger (1888) also furnish data.

*Bi-trochanteric breadth.*—This width is not plotted in chart J, but data are given in table K. Godin's data show this width to be about 14 per cent greater than the bi-crestal width at age 13.5 and 16 per cent greater at age 17.5. Quetelet (table E) gives data on the bi-trochanteric width from infancy to maturity. The widths tabulated are relatively considerably greater than those of Hall (1896) for boys of ages 9 to 23 and are slightly greater than those of Godin (1910). The data given in table K are based on relatively few measurements, but fit in well with Hall's data and with the American college data. In the latter part of childhood and subsequently girls have a greater bi-trochanteric width than boys. For variations in young adults based on stature and maturity the reader may consult table L.

#### GIRTHS.

Some of the chief girths are plotted in chart J and tabulated in table K.

*Girth of head.*—The curves in chart J are based primarily on data from Schmid-Monnard (table A) for infancy and on data from Hastings (table F) for the period from 5 to 20 years of age. Use has also been made of data from Quetelet (table E), Landsberger (1888), MacDonald (1898), Daffner (1902), Hall (1896), Ernst (1906), Schwerz (1910), and other investigators. The largest relative head-girth reported as normal for infants is that by Orsehansky, 70.9 per cent of the stature for females. Quetelet gives comparatively low figures, 67.0 per cent for males. Schmid-Monnard gives 68.8 per cent for males, 67.1 per cent for females. For the period of childhood and adolescence the data given by the various authors mentioned are remarkably similar if one takes into consideration the diversity of material. We can not here enter into a discussion of the divergences in the reported data. The girth of head steadily declines with growth in stature. The girth of the female head is slightly smaller than that of the male head, although at the time of puberty in girls boys

of the same age and stature may have a slightly smaller head-girth. In adults relative head-girth varies inversely with stature (table L).

*Girth of neck.*—Data on the girth of the neck are given by Quetelet (1870), Landsberger (1888), Hall (1896), and Godin (1905). According to Quetelet, it is 29.7 per cent of the stature in the new-born, 20 to 20.3 per cent in the adult male, 19.2 to 19.4 per cent in the adult female. It is relatively smallest in the period immediately preceding puberty (males, 18.9 per cent). It is slightly smaller in females than in males.

*Girth of shoulders.*—Quetelet (1870) gives the girth at the level of the acromion processes as 64.2 per cent of the stature in infants; it is least in the period just preceding puberty, males 52.1 per cent, females 51.5 per cent; subsequently it increases to 55.3 per cent at the age of 20 in men, 53.7 per cent in women, and 55.8 per cent at the age of 30 in men, 54.2 per cent in women.

*Girth of chest.*—There is a large amount of material on chest-girth, but much of it is unsatisfactory, owing to the difficulty of selecting and applying uniform methods of measurement. We can not here enter upon a discussion of this broad subject. The curves in chart J are based primarily on the data of Schmid-Monnard (table A) for infancy and early childhood and on those of Hall (1896) for later childhood and adolescence. Quetelet (table E) and Weissenberg (table D) give data on chest-girth from infancy to maturity, other investigators for more limited periods of growth. Among these other investigators may be mentioned Roberts (1878), Pagliani (1879), Kotelmann (1879), Landsberger (1888), Porter (1894), Moon (1892), Hitchcock (1900), Daffner (1902), Barr (1903), Godin (1903), Rietz (1903), Ernst (1906), and Seaver (1909). The relative chest-girth is largest in infancy. It is then about two-thirds of the stature. It is smallest in the period preceding puberty, when it is considerably less than half the stature. During adolescence it usually reaches about half the stature in males, less frequently in females. In the adult it usually continues to increase in size for some years. In the adult it is larger in short than in tall individuals (table L).

*Girth of waist.*—Data on the girth of the waist are given by Quetelet (table E), Landsberger (1888), and Hall (1896). The relative girth declines from infancy to the period preceding puberty and then begins to increase. The increase may continue until the tenth decade or later. It is larger in short than in tall men, but the reverse seems to be the case for young women (see table L).

*Girth of pelvis.*—This measurement is usually taken at the level of the anterior superior iliac spines. Data from Quetelet (table E) show an increase of relative girth from infancy to early childhood and then a decrease to the period preceding puberty, followed by a marked increase during adolescence and early adult life. The female girth for a given stature, according to Quetelet's figures, is slightly less than the male up to the period of puberty, but after this period it greatly exceeds the male. For men aged 30 Quetelet gives it as 47.5 per cent of the stature, for women as 53.0 per cent. Hall (1896) has given data on this measurement for American boys 9 to 23 years of age. His figures for relative girth are higher than those of Quetelet, but were taken at a lower level.

*Girth of hips.*—This measurement, taken at the level of the trochanters, is a customary one in American colleges, but there appear to be few data on it for infancy and childhood. Even Quetelet (1870) leaves blank the column reserved for it. Moon (1892) gives some data on American school-boys. In table L may be found a summary of some American college statistics. These data show that this girth is relatively greater in short than in tall individuals and in the mature than in the immature of a given stature. They do not show much sexual difference for a given stature.

*Bone-girths.*—Measurements of the girth of the forearm just above the wrist, of that of the leg just above the ankle-joint, and of that of the knee-joint or of the extremity near the knee-joint are useful in giving data on growth of the long bones in girth. As an example of measurements of this character, we give, in table E, Quetelet's data on the relative girth of the knee-joint from infancy to maturity. He shows a decrease from 22.8 per cent in the new-born to 20.5 per cent at the age of 13 in males, 19.9 in females, and an increase during adolescence to 20.6 per cent in males, 21.1 per cent in females. Hall (1896) shows a slight decrease in relative knee-girth preceding puberty followed by a subsequent increase. In the adult (table L) short men show a relatively greater knee-girth than tall men. For women this is not evident. Women show a greater knee-girth than men of the same stature. Quetelet's data on the diameter of the lower part of the forearm show a decrease in relative girth from 15 per cent of the stature at birth to 9.4 per cent at the age of 12 in the male, 9.0 per cent in the female. It increases during adolescence to 9.9 per cent in the male, 9.3 per cent in the female. Short male college students show a greater relative wrist-girth than tall students (table L). Moon (1892), Hall (1896), and Godin (1903) give data on the growth of the wrist. The girth of the leg measured just above the ankle-joint, according to Quetelet's figures (1870) for women, shows a course of development similar to that of the forearm, 16.3 per cent in the infant, 12.1 per cent preceding puberty, 12.7 per cent in the young adult. Hall's data for men (1896) correspond well with those of Quetelet's for women. There appears to be some mistake in Quetelet's data for this measurement in males at the time of puberty.

In the development of the skeleton of the limbs it appears evident, therefore, that relative girth decreases during childhood, increases during adolescence, and is stationary in the adult. In those who reach definitive stature relatively early the girths of the limb skeleton are of the same relative size as in mature young adults (table L).

*Muscle-girths.*—The girths of the arm, upper part of the forearm, the thigh, and the calf give a good index of growth of muscles, although they are all influenced by deposit of fat as well as by muscular development. In table E data from Quetelet (1870) are given on the relative girths of the arm, thigh, and calf from infancy to maturity. All show a decrease from infancy to the period preceding puberty and an increase during adolescence and early adult life up to 30 or 40 years of age. During the first year after birth, according to Quetelet's data, there is a growth in the relative diameters mentioned as well as of the girths of the knee-joint and of the

leg above the ankle-joint, but not of the forearm. Moon (1892), Hall (1896), Ernst (1906), and Godin (1910) give data on the girths of the arm, forearm, thighs, and calves in school children which, while differing in details, in general confirm the course of growth by Quetelet's data. According to the data of Ernst, the female thigh begins to be larger than that of the male as early as the eighth year, while Quetelet's data do not show this to be the case before the period of puberty.

Table L shows that in college students young individuals have smaller muscle-girths than mature individuals of the same stature while the bone-girths are of the same relative size. Short men have relatively larger muscle-girths than tall men, but in women this is not shown. Women have relatively larger thighs and calves, smaller arms and forearms, than men.

### GROWTH AND THE HEIGHT-WEIGHT INDEX OF BUILD.

The foregoing review shows clearly that there is a close correlation between stature and build and that build varies in definite directions as stature increases and after full stature is reached. These changes are reflected in the height-weight index of build.

*Height-weight index growth-curve.*—If a given individual were weighed and measured periodically from birth to old age and the height-weight indices were calculated from these measurements and plotted, we should have a curve of growth that would give a much clearer picture of the change in the proportions of his body than we could obtain by plotting curves of weight and stature independently. If we had a large number of such curves we could make use of them for plotting growth-curves typical for the majority of individuals. As it is, we have data for plotting individual growth-curves of this character merely for limited periods in the life-cycle (see charts E, F, G, and H) and are forced to plot typical growth-curves from averages of height and weight of large numbers of individuals grouped according to age. Typical growth-curves of height-weight indices of build based on data of this kind for the whole life-cycle with especial reference to the average American are shown in chart A and have also been reproduced in charts I, J, and K. The data on which these curves are based are too voluminous for publication here. I shall therefore give merely some of the more important points concerning the growth-curve as a whole and its relations to the chief periods of the life-cycle.

In chart A a series of indices is given beginning with 1.000 at the left and extending to 0.400 at the right. Beneath each index is a series of numbers to express the weight in pounds of individuals of the stature shown in the column at the left. All individuals in a given column have the same index, but with each additional inch of stature there is a definitely associated addition to the weight which increases with the stature. Thus, in the 0.700 column there is an addition of 3.5 pounds to the weight as one passes from a stature of 28 inches to one of 30 inches, while there is an addition of 23 pounds as one passes from a stature of 74 inches to one of 76 inches. The indices and corresponding weights refer to the center of the columns in which they lie. The lines separating the columns represent intermediate quantities. Thus the line between the 0.700 and 0.650 column represents an index

CHART A.—*Typical Height-weight-index curves birth to maturity*

Stature, inches.	Weight in pounds at a given stature and at an index of—													Typical age in years and months Males
	1.000	.950	.900	.850	.800	.750	.700	.650	.600	.550	.500	.450	.400	
76 0	439	417	395.	373	351.	329.	307.	285.	263.	242.	220	198.	176.	grs. mos.
74 0	405	385	365.	341	324	301	284	263.	243	223	203	182	162.	
72 0	373.	355	336.	317.	299	280	261.	243.	224	205	187	168	149.	
70 0	343 3	326	309.	292	274	257	240.	223.	206	189	172	154.	137.	
68 0	314	299	283.	267	252	236.	220.	204	189	173	157	141	126	18+
66 0	288	273	259.	244.	230	216	201	187	173	158.	144	129	115	16 6
64 0	262	249	236	223	210	197.	184.	170	157	144	131	118	105	15 6
62 0	238	226	215.	203	191	179	167	155	143	131.	119	107	95 3	14 8
60 0	216	205	194.	184	173	162	151	140	130	119.	108.	97 2	86 4	14
58 0	195	185	176.	166.	156	146.	132	127	117	107	97 6	87 8	78	13 2
56 0	176	167	158.	149.	140	132	123	114	105.	96 6	87 8	79	70 2	12 3
54 0	158	150	142.	134	126	118	110	102	94 5	86 6	78 8	70 9	63	11 3
52 0	141	134	127.	120	113	106	98 4	91 4	84 4	77 3	70 3	63 3	56 2	10
50 0	125	119	113	106	100	93 8	87 5	81 3	75	68 8	62 5	56 3	50	9
48 0	111	105	99 5	94	88 5	83	77 4	71 9	66 4	60 8	55 3	49 8	44 2	8
46 0	97 3	92 5	87 6	82 7	77 8	73	68 1	63 3	58 4	53 5	48 7	43 8	38 9	7
44 0	85 2	80 9	76 6	72 2	68 1	63 9	59 6	55 4	51 1	46 8	42 5	38 3	34 1	6
42 0	74 1	70 4	66 7	63	59 3	55 6	51 9	48 2	44 5	40 7	37 1	33 3	29 6	5
40 0	64	60 8	57 6	54 4	51 2	48	44 8	41 6	38 4	35 2	32	28 8	25 6	4 4
38 0	54 9	52 1	49 4	46 6	43 9	41 2	38 4	35 7	32 9	30 2	27 4	24 7	21 9	3 6
36 0	46 7	44 3	42	39 7	37 3	35 3	32 7	30 3	28.	25 7	23 3	21	18 7	2 10
34 0	39 3	37 3	35 4	33 4	31 4	29 5	27 5	25 5	23 6	21 6	19 7	17 7	15 7	2 2
32 0	32 8	31 1	29 4	27 9	26 2	24 6	22 9	21 3	19 7	18	16 4	14 7	13 1	1 8
30 0	27	25 7	24 3	23	21 6	20 3	18 9	17 6	16 2	14 9	13 5	12 2	10 8	1 2
28 0	22	20 9	19 8	18 7	17 6	16 5	15 4	14 3	13 2	12 1	11	9 88	8 78	9
26 0	17 6	16 7	15 8	14 9	14 1	13 2	12 3	11 4	10 5	9 68	8 8	7 92	7 04	5
24 0	13 8	13 1	12 4	11 7	11 1	10 4	9 66	8 97	8 28	7 59	6 9	6 21	5 52	3
22 0	10 7	10 1	9 63	9 1	8 56	8 03	7 49	6 96	6 42	5 89	5 35	4 82	4 28	1
20 0	8.	7 6	7 2	6 8	6 4	6	5 6	5 2	4 8	4 4	4	3 6	3 2	B

of 0.675. The space between the lines on each side of the 0.700 column may be considered as extending from index 0.675 to 0.725 and divisible correspondingly. In the same way the weight in pounds represented at any given level in a given column refers to the center of the column at that level and the points on either side of this represent weights intermediate between the weight given and the neighboring weights on each side. Furthermore, points between the base of the line for a given stature and corresponding series of weights and the base of the next line represent intermediate statures and corresponding intermediate weights. Thus, a point midway between the base of weight 68.1 in the 0.700 column and weight 77.4 in the same column would represent a stature of 47 inches and a weight of 72.8 pounds. In a chart of this kind it is possible, with fair accuracy, to plot to stature and weight and read off the corresponding index at the top, or to plot to stature and index and estimate the corresponding weight from the weights near the point plotted. Typical growth-curves for males and for females are plotted in this chart. The curve for males begins with index 0.918 in the 0.900 column at a stature of 20 inches. It takes a vertical course to the level of stature 27 inches and then swings in a parabolic curve to the left, index decreasing as stature increases. The apex of the parabolic curve is reached at stature 56 inches, index 0.413, but soon after the apex is passed the curve changes its character and takes a nearly vertical course until just before full stature is reached, when it curves sharply to the left and finally terminates in a straight line. This portion of the curve represents the increase in weight and index which takes place from the time full stature is reached until the age of 40 or 50 years. In old age there is usually a decrease in stature which would cause the line to curve downwards and a decrease in weight which would cause it to hook to the right. Lack of adequate data, however, has prevented us from plotting this portion of the curve. The curve for females follows closely that of males until the apex of the parabolic curve is reached. The parabolic curve is continued further beyond the apex and the curve to the left appears earlier in women than in men.

The age at which a given point in the height-weight index growth-curve is reached is a variable factor. At the right of the chart, however, the approximate age is indicated at which the average healthy American male reaches the stature shown at the corresponding level at the left of the chart. For females the age at which a given stature is reached varies somewhat from that for males (tables H, I, and J).

Unfortunately it is not possible to construct a simple chart which can be used both for the centimeter-gram units and for inch-pound units. A chart similar to chart A may, however, be readily constructed on the metric system. The curves are the same if the proportions of the chart are preserved. As pointed out above, the metric system index may be calculated from the inch-pound index by multiplying the latter by 0.02768.

*Weight-for-height curves.*—As opposed to the height-weight-index growth-curve, based on averages, and intended to show a typical course of development in stature and build, I have designated as *weight-for-height* curves, curves which illustrate the variation in build shown by a group of individuals of approximately the same

age but who vary in stature. For each subgroup the average or mean weight is divided by the cube of the stature characteristic of the subgroup. From the empirical data thus obtained it is possible to calculate curves to illustrate the weight and index typical of a given stature at a given age. Curves of this kind are shown in charts B, C, D, and H. Weight-for-height curves are hyperbolic in character.

We may now consider some of the characteristic features of curves based on the height-weight index of build in infancy, childhood, adolescence, and maturity.

For *infancy*, data on height in relation to weight are scanty except for the new-born. Such data as exist are somewhat contradictory. After plotting the available data I have come to the conclusion that for the present it is best to consider the height-weight index for this period as a straight line with a uniform value of 0.918 (0.915 for females) for any given stature from 20 inches to 27 inches (26.5 inches for females). The choice of 0.918 as the value of the index is purely empirical and can be considered only as a rough approximation. As such, however, it is of value, because wide departures from it enable us to judge whether a child is abnormally thin or abnormally fat, and, within limits, to what extent. In the use of 0.918 as a standard height-weight index for this period (0.02543 is the corresponding centimeter-gram ratio), the following facts should be taken into consideration:

1. At the time of birth full-term short infants are relatively heavy; long infants are relatively light.
2. Growth, during infancy, even in healthy babies, is seldom uniform. Periods of rapid growth in length are apt to alternate with periods of less rapid growth in length and a relatively more rapid growth in weight.
3. Statistics relating to groups of healthy infants as a rule show a relatively large body-weight when the measured length is relatively short for age and vice versa.
4. To some extent differences in height-weight indices based on the observations of different investigators are to be attributed to variation in method of measuring length.

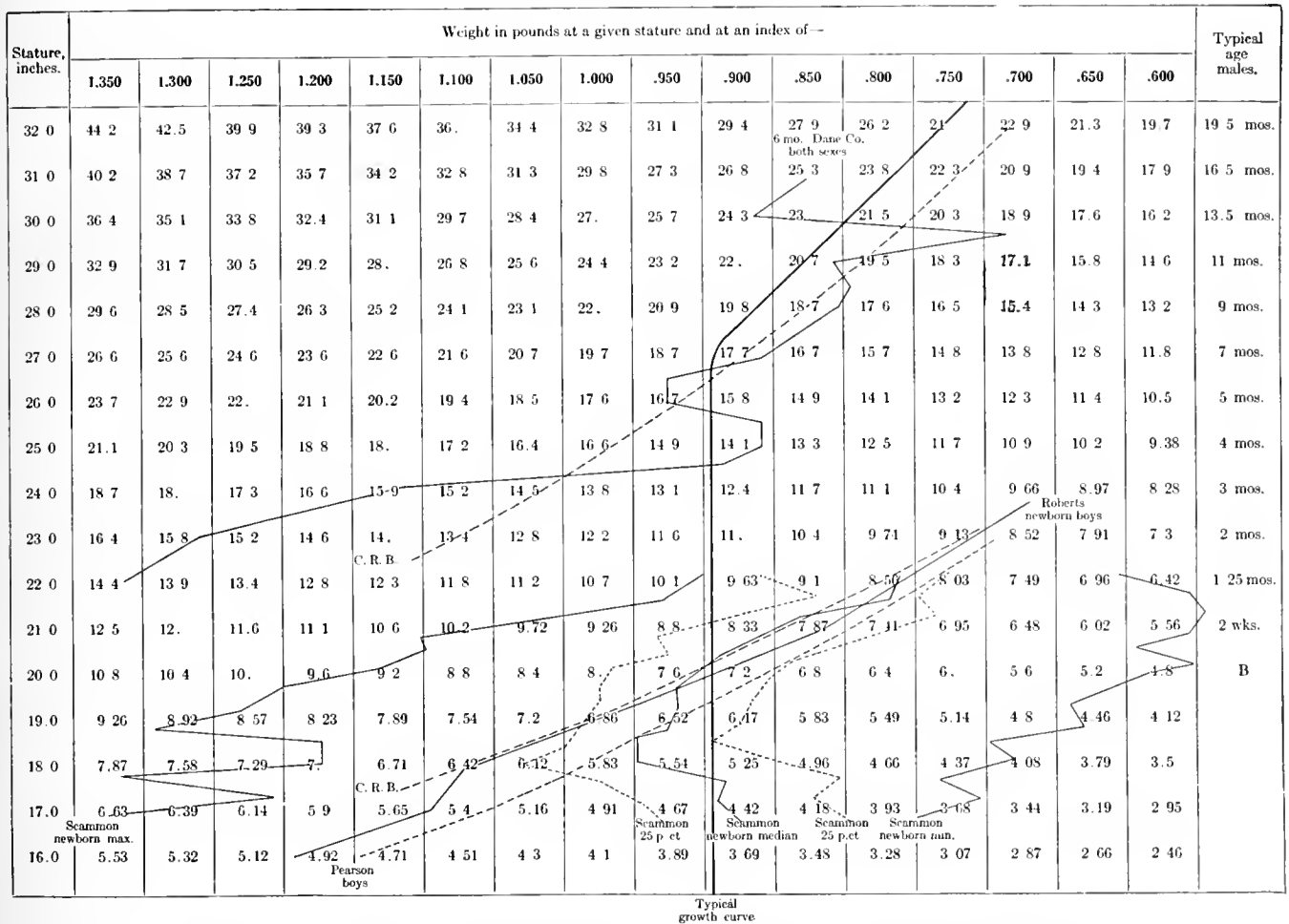
The index 0.918 appears to be approximately normal for an infant 20.3 inches (51.56 cm.) long at birth, a length which may be taken as normal for American male children. Holt (1916) gives a slightly greater length (20.61 inches, 52.3 cm.), Taylor (1918) a slightly less length (51.18 cm.). Infants shorter than this, as a rule, have a higher index, *i. e.*, are relatively heavier; those longer are relatively lighter. Thus the male infants of the British Anthropometric Report 1883 with the average length of 19.52 inches (49.58 cm.) have an index of 0.957 (metric 0.0265), while the male infants of the Pearson series (1899), with a mean length of 20.503 inches (52.08 cm.), have an index of 0.847 (metric 0.02344).

In chart B several curves are given to illustrate the relative proportions of the body in the new-born. The general construction of this chart is like that of chart A and therefore need not be described.

The heavy line which extends upwards at the left side of the 0.900 column as far as the 27-inch-stature line and then swings toward the right represents the



height-weight-index growth-curve which we have chosen as typical of the healthy American male baby. Up to the seventh month (the period of infancy as defined above) it represents an index of 0.918. Here we have illustrated rapid growth in length and weight without change in the proportions of the body. While we thus represent the type-curve for this period as a straight line we recognize, of course, that there are some changes during this period in the general proportion of the body in all individuals and marked fluctuations in many individuals. Further data will enable the construction of a more accurate type-curve for this period, but the period

CHART B.—*Weight for height curves at birth and six months of age.*

is undoubtedly marked by relatively slight change in bodily proportions. The data of Ahlfeld (1871) indicate a similar condition and a similar index during the latter part of fetal life.

In the report of the British Anthropometric Committee (1883), drawn up by Roberts and Rawson, a table is given showing the average weight for a given stature in inches for each sex at birth based on a study of 451 males and 466 females. A curve based on the data for the male infants is designated *Roberts, new-born boys*, in chart B. It will be noted that the shorter infants are here markedly heavier

for stature than the taller infants. The curve for girls (not shown in the charts) is similar. K. Pearson (1899) gives a summary of results of a study of 1,000 male and 1,000 female babies born at "the normal period" at the Lambeth Lying-In Hospital. He gives formulæ for computing the probable weight from a given length in each sex. A curve plotted from his formula for males ( $W = 0.553 L - 4.04$ ) is represented by a dotted line labeled *Pearson, boys*, in chart B. This curve likewise shows short infants relatively much heavier than tall infants, but throughout lighter than those in the first series. The curve for female infants is similar.

Dr. R. E. Scammon, of the University of Minnesota, has been making an extensive study of available literature on the stature and weight of infants at birth and has plotted weight for stature for over 1,800 infants of both sexes, whose measurements he has found recorded in the literature, chiefly of western Europe. He has kindly placed his data at my disposal and from these data I have plotted the curves labeled *Scammon* in chart B. At the left, labeled "*new-born maximum*," there is plotted a curve which shows the heaviest infant for each centimeter of height from 43 to 56 cm. This curve, though irregular, shows a relative decrease of weight with increase of stature. Beginning at the left of the typical growth-curve, a curve represented by dotted lines labeled "*25 per cent*" represents the mean of the heavier half of the infants for each centimeter of stature. Here it will be noted that the shortest infants are relatively light, but those above 46 cm. (18.1 inches) show in general a relative decrease in weight with increase in stature. The curve of *median weight*, which begins near the typical growth-curve, takes a similar course, as does also the dotted *25 per cent* curve to the right of this. Between the two 25 per cent curves are included 50 per cent of Scammon's cases for each centimeter of stature. Still further to the right the "*new-born minimum*" curve represents the smallest weight for a given stature found by Scammon.

It will thus be seen that while the shortest of the infants recorded by Scammon are grouped about the typical growth-curve, as we would expect them to be if premature rather than full-term (Scammon, however, endeavored to exclude such infants), new-born infants over 18 inches in length show a relative decrease in weight with increase in stature.

The curve labeled *C. R. B.*, beginning in the 1.150 column at stature 17.5 and marked by a broken line, represents a type curve which I have constructed for new-born male infants based on the data cited and other data. The formula for this curve is  $\text{index} = \frac{27.000}{x-1} - 0.485$ , where  $x$  = stature in inches. For females the corresponding curve is  $\text{index} = \frac{30.000}{x-1} - 0.664$ .

For age 6 months I have a limited amount of data based on statistical studies made in Dane County for the investigations now being carried out on height and weight of children by the Children's Bureau of the U. S. Department of Labor, Robert E. Woodbury, director of statistical research. The curve in chart B, labeled *6 mo. Dane Co. both sexes*, is based on a study of records of 60 individuals of both sexes. The small number of data available makes the curve irregular, but it shows a similar relatively large weight for short, small weight for tall individuals at this

age. The curve labeled *C. R. B.* is based on data for this and neighboring months of age and represents a tentative type curve for males, the formula for which is  $\text{index} = \frac{30.000}{x-1} - 0.258$ . It may be noted that the curve takes a course somewhat similar to that of the typical growth-curve of childhood, although it is a hyperbolic curve, while the latter is a parabolic curve. Further details and illustrative tables relating to the period of infancy are reserved for future publication. From the data presented it is evident that, as a rule, an infant which grows unusually fast in length is apt to be relatively thin, while an infant which grows slowly in length, if healthy, is apt to put on weight. What the limits of normal bodily proportions in infancy are we can not tell from the evidence at hand, but they probably vary from those represented by an index of 0.700 (metric 0.01938) for unusually long infants to 1.200 (0.03322) for short infants. The anatomist in studying the structure of infants should record length, body-weight, and the height-weight index as well as age. The body fat probably varies in quantity more than other structures in the body, so that in studying relative weight of organs it is important to know whether or not we are dealing with infants of average adiposity.

The *period of childhood* extends from infancy to adolescence. It is characterized by well-defined curves of change in relative proportions of the body and in the height-weight index of build. From the standpoint of average age it extends from the sixth or seventh month of infancy to the fourteenth or fifteenth year. From that of average dentition it extends from the eruption of the first incisors to that of the second molars. From that of the height-weight index it comprises a period during which alterations in the index approximate a simple parabolic curve which for inch-pound units and for boys may be expressed as  $\frac{413 + 0.6(x-56)^2}{1000}$ , where  $x$  = height in inches, and for girls as  $\frac{410 + 0.6(x-55.5)^2}{1000}$ .

Similar curves may be used to express centimeter-gram ratios in terms of the *percentage* found by dividing the weight in grams by the cube of height in centimeters; formulæ for these curves are for boys  $1.1432 + 0.000258(x-142)^2$ , for girls  $1.1349 + 0.000258(x-141)^2$ . The curves are based on a study of all available data relating to average weight and average stature at a given age during childhood. Weight is taken as weight without clothes. Where weight in clothes is given, deduction for estimated weight without clothes has been made. The data relating to this study are too voluminous for publication here. The figures on which are based the growth-curves of childhood, as well as of infancy and adolescence and maturity, are given in tables H, I, and J. Charts C and D (constructed in a manner similar to chart A described on p. 519) illustrate both the height-weight index growth-curves here described and weight-for-height curves.

The height-weight-index growth-curves are proposed as type curves of indices of build during childhood in healthy Americans. With them we may compare indices of given individuals or groups of individuals for the sake of estimating their relative build. A low index at a given stature in an undeformed individual indicates a thin body; a high index indicates a stocky and usually a fat individual. The heavy lines drawn through charts C and D represent the typical growth-curve

for males. Estimated average age for well-developed individuals is indicated at the right side of each chart. The light lines represent the curve of average indices for a given height at a given age "weight-for-height curves." The numeral at the upper end of each curve signifies the age in years.

The parabolic height-weight index-growth curve for boys has its vertex, point of lowest height-weight index, at height 56 inches (approximately 142 cm.). This would indicate that at about this height the average healthy American boy is lightest in relation to his stature. According to our estimates he is 12.25 years old at this period. To the left the curve continues smoothly to the period of infancy at 27 inches stature. It is, of course, not probable that a sharp break in the growth curve from a parabola to a vertical line occurs at this point. In the charts we have slightly rounded the angle of transition. We must have much more extensive data than are at present available before we can construct a normal curve of transition from the infantile growth-curve to that of childhood. For girls I have empirically selected a growth-curve the vertex of which is shifted 0.5 inch (1 cm. in the curve of the centimeter-gram index) below that for boys. This gives a difference of indices which approximates that existing between the two sexes. We can not draw curves which show sexual differences with perfect accuracy until we have a far greater amount of data on weight without clothes in the two sexes. The curve for girls meets the infantile curve at a height of 26½ inches.

Chart C shows weight-for-height curves for ages 5, 9, 11, and 13 years. It is to be noted that at 5 years the weight-for-height curve, although hyperbolic instead of parabolic in character, for the range of variations in stature found at this age, very nearly coincides with the growth-curve. This means that a short child of 5 will have very nearly the same height-weight index as a younger child of the same stature, and a tall child of this age very nearly the same proportions as an older child of the same height. At 9 years of age the shorter children average slightly heavier, the taller slightly lighter than younger and older children of respectively the same stature. On the other hand, the weight-for-height curves at ages 11 to 13 show that the shortest children

CHART C.—Weight for height curves of boys 5, 9, 11 and 13 years of age.

Stature, inches.	Weight in pounds at a given stature and at an index of—						Typical age in years.
	.650	.600	.550	.500	.450	.400	
55.0	108	99.8	91.5	83.2	74.9 13 yr.	66.6 11 yr.	58.2
54.0	102	94.5	86.6	78.8	70.9	63.0	55.1
53.0	96.8	89.3	81.9	74.5	67	59.6	52.1
52.0	91.4	84.4	77.3	70.3	63.3	56.2	49.2
51.0	86.3	79.6	73	66.4	59.7	53.1	46.4
50.0	81.2	75	68.8	62.5	56.3	50.5	43.8
49.0	76.4	70.6	64.7	58.8	52.9	47	41.2
48.0	71.9	66.4	60.8	55.3	49.8	44.2	38.7
47.0	67.5	62.3	57.1	51.9	46.7	41.5	36.3
46.0	63.3	58.4	53.5	48.7	43.8	38.9	34.1
45.0	59.3	54.7	50.2	45.6	41	36.5	31.9
44.0	55.4	51.1	46.8	42.6	38.3	34.1	29.8
43.0	51.7	47.7	43.7	39.8	35.8	31.8	27.8
42.0	48.2	44.5	40.7	37.1	33.3	29.6	25.9
41.0	44.8	41.4	37.9	34.4	31	27.6	24.1
40.0	41.6	38.4	35.2	32	28.8	25.6	22.4

Typical  
growth curve

are lighter than younger children of the same stature. These weight-for-height curves are based primarily on the excellent study of Ethel M. Elderton (1914) on the height and weight of Glasgow school children. In her tables the phenomena just mentioned are clearly brought out, although she does not call attention to them in the text. They indicate that children of this age markedly retarded in growth in stature are still more retarded in weight, and that disease or profound physical alterations must be at play. At all other periods groups of individuals retarded in stature tend to some extent to put on extra weight. This is especially noticeable after puberty. On the other hand, although children in whom growth in stature is accelerated do not generally grow equally fast in weight, there are many exceptions at all ages. This is shown especially in the growth-curves of several of the more rapidly growing children studied by Baldwin.

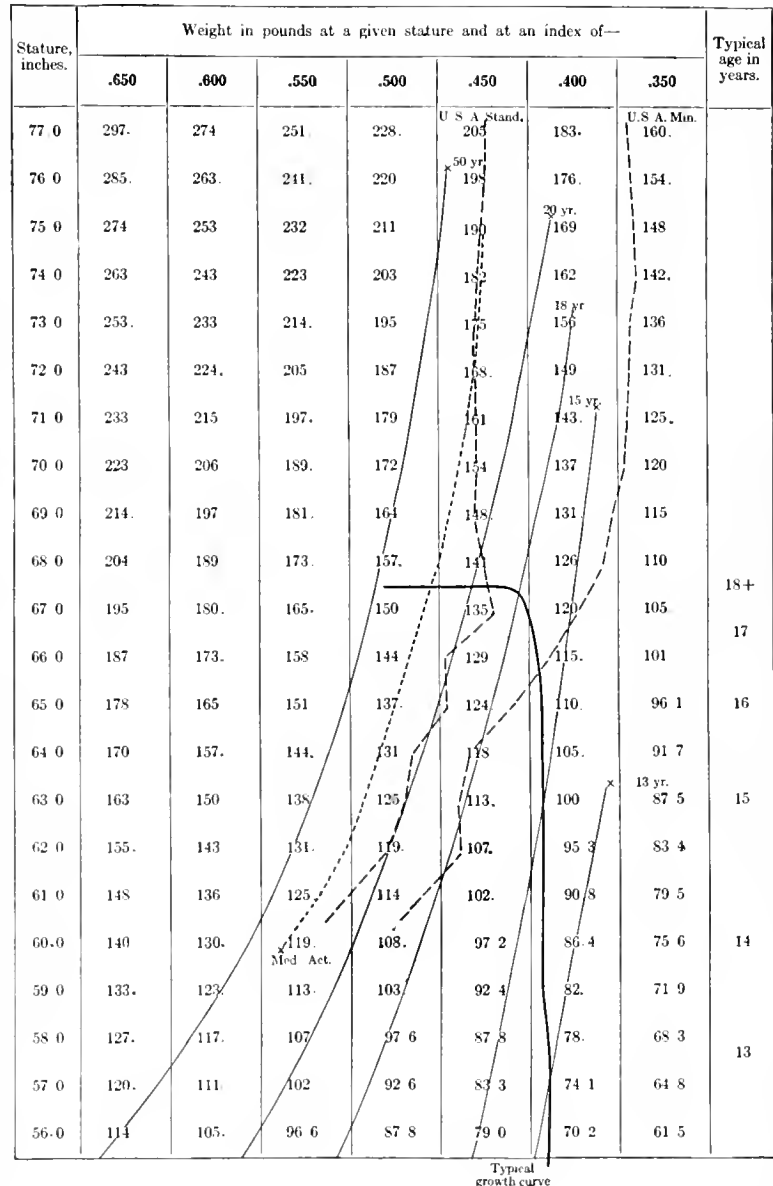
*Adolescence.*—Above the vertex the parabola for girls is continued to a height of 63 inches at the age of 16. It is very difficult to estimate from available data a curve which may be considered normal for a girl who attains a stature of 63 inches. Girls who will reach a full height of less than 63 inches and girls who will attain more than average stature are averaged together in age-group statistics in such a way that it is impossible to trace accurately the course of development of the average girl who reaches this stature. For an accurate study of this period we need a large number of measurements of individuals from 10 to 20 years of age. Baldwin's studies (1914), while of great value, are based on too small a number of individuals to assist us greatly in this connection, and the same is true of the few other studies on the growth of individuals at this period. For the present the continuation of the parabola of height-weight indices characteristic of childhood up to the period when full height is nearly reached in women seems best to correlate the data at hand. This I have done in constructing the curve of height-weight indices for girls. After the age of 16 the average girl increases very slightly in height, but increases markedly in weight. The growth-curve, therefore, loses its parabolic character and becomes a horizontal line. Empirical estimates of indices for successive age-periods succeeding 16, based on all available height-weight data, are given in table J.

For American boys the part of the growth-curve above the vertex of the parabola takes quite a different course from that characteristic for girls. For a short period, up to height 59 inches (age 13.5 years in the average healthy boy), the parabola is continued beyond the vertex. There then ensues a period apparently lacking in the growth-curves of most girls, during which the height-weight index remains nearly stationary in spite of very rapid growth in stature. The beginning of this period coincides with the time when (according to Crampton) pubescence is most frequent in boys, and extends to about the age of 16. Crampton has pointed out that the first part of the post-pubescent period is that of most active growth in boys. The period in question is one of very active growth in height. As growth in stature slows down slightly in the sixteenth to seventeenth year, there is a relative gain in weight. This does not appear in individuals who are relatively retarded in growth and whose period of most active growth from the chronological standpoint is prolonged beyond that just described. Such individuals may have a relative

decrease in index at about the sixteenth year. After the age of 17 in most boys growth in weight is relatively more rapid than growth in height, and after 18 (except in those retarded in development) growth in stature is relatively slight, so that the height-weight growth-curve soon becomes horizontal. In table J a series of empirical height-weight indices is given for successive age-periods from 18 to 50 years for individuals of 67.5 inches height. Tall individuals continue a low height-weight index for a longer period than short individuals; short individuals increase in height-weight index much more rapidly than those of average build.

*Maturity.*—Adolescence passes gradually into maturity. Growth in stature in most individuals is nearly completed before the age of 20, although in some individuals it is continued for several years beyond this age. We may consider the period of maturity to begin when full stature is reached. The male height-weight growth curve for this period and weight-for-height curves for ages 13, 15, 18, 20–24, and 50+ years are shown in chart D. It is to be noted that with increasing age the shorter individuals become relatively much

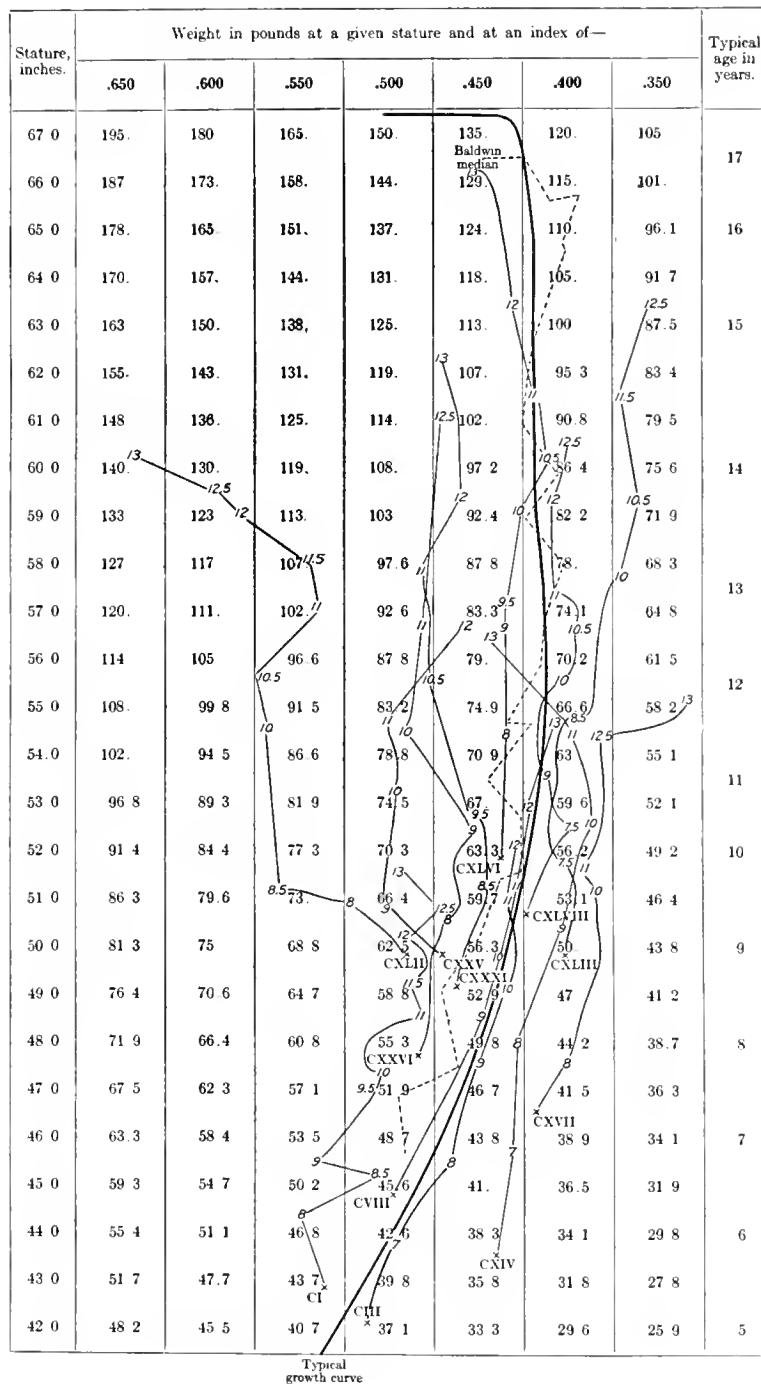
CHART D.—Weight-for-height curves of males 13, 15, 18, 20 and 50 years of age; Medico-Actuarial curve average male; U. S. army standard and minimum curves.



heavier than those of average build. The curve labeled “medico-actuarial,” shown by a dotted line is based on average weight for a given stature irrespective of the age of the insured. The statistical tables of the medico-actuarial mortality investigation (1912) were utilized for plotting this curve, as well as those for weight-

for-height in adults referred to above. Allowance has been made for height of heels in indicating stature and for weight of clothing in indicating weight. The curve

CHART E.—Height-weight-index curves of individual boys 6 to 13 years of age.



very nearly coincides with the weight-for-height curve for ages 30 to 34 years, which is approximately the average age of the insured. The curves of the United States Army standards of weight-for-height are represented in the table by dashes. For

CHART F.—Height-weight-index curves of individual girls 6 to 13 years of age.

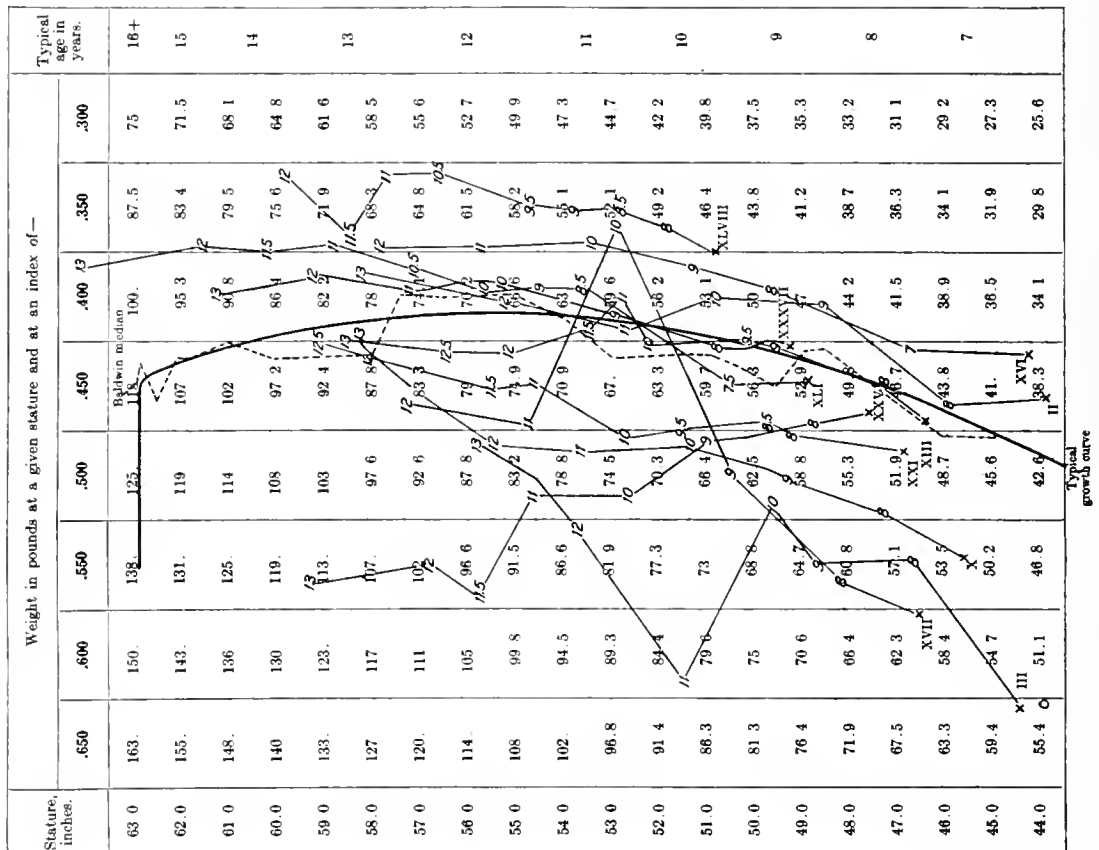
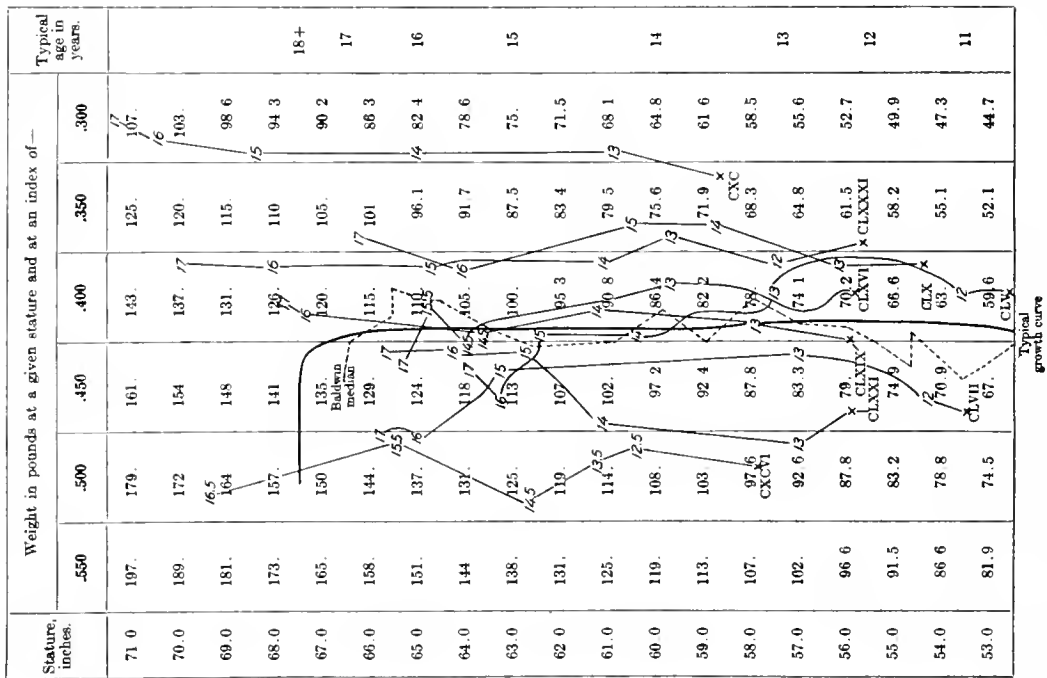


CHART G.—Height-weight-index curves of individual boys 11 to 17 years of age.

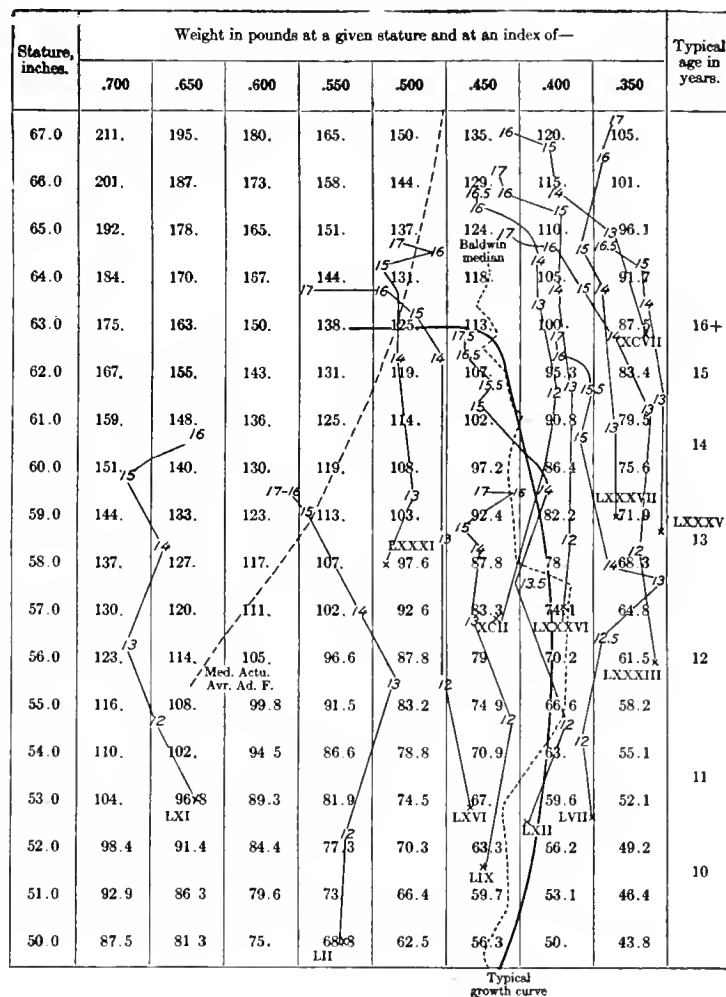




individuals below average stature the army normal standard corresponds fairly well with our standard curve for age 20 years, while for individuals above average stature it corresponds with our standard curve for age 30 years. The curve for the army minimum standard is irregular.

*Individual growth-curves* are illustrated in charts E, F, G, and H, which are similar in general construction to those described above. The heavy line in each chart represents the typical height-weight-index growth-curve. The ages given in

CHART H.—Height-weight-index curves of individual girls 11 to 17 years of age.



curve. The women, however, reach a stature of 63.8 instead of 63 inches.

Height-weight-index growth-curves are plotted for a number of individuals tabulated by Baldwin in his tables 4, 5, and 13 to 22. Each individual is distinguished by a number. These numbers run from 1 to 100 for girls, 101 to 200 for boys. The number of each individual selected for plotting in my charts is shown by a Roman numeral at the base of his curve of growth. Along each curve of growth Arabic numerals are placed which indicate the age at which a given stature and weight was

reached and the corresponding height-weight index. Each curve begins one year earlier than that indicated by the first Arabic numeral along the course of the curve. Thus, in chart H, girl LXI at 11 years of age had a stature of 134.8 cm. (53.1 inches), weighed 96.5 pounds, and had an index of 0.646; at 12, stature 139 cm. (54.7 inches), weight 110 pounds, index 0.671; at 13, stature 143 cm. (56.3 inches), weight 122.8 pounds, index 0.688; at 14, stature 148 cm. (58.3 inches), weight 132 pounds, index 0.666.

It is to be noted in these charts that the individual growth-curves correspond fairly well with typical growth-curves based on averages. This would be still more striking were it practical to plot the curves of all of the individuals tabulated by Baldwin, but so many of the curves fall along the line of the typical growth-curves that the picture becomes too confusing for the purpose of reproduction. The curves selected, therefore, illustrate individuals of varied types, including the most extreme.

Fat individuals tend on the whole to remain short and to grow relatively fatter with age. Tall individuals tend to be thin until growth is completed and to have growth-curves which parallel the typical growth-curve.

In addition to the data shown in the other tables of this series a curve marked by dashes is shown in chart H to illustrate the average weight for a given height found, irrespective of age, in the women tabulated in the medico-actuarial investigations. Allowance has been made in the chart for heels and for weight of clothes. Short women are very much heavier than growing girls of the same height, while the difference is slight between tall women and tall girls at the time they cease to grow in stature.

#### TRANSVERSE SECTION AND TRANSVERSE DIAMETER INDICES.

In charts I, J, and K the curve labeled *height-weight index* represents the height-weight-index growth-curve and enables one to compare the relations which exist between weight and stature with those which exist between several linear and volumetric measurements and stature during growth in height. Only that part of the curve for females is shown which diverges markedly from that of the male. This is shown by broken lines. The height-weight index is primarily an expression of the relation between the volume of the body stated in terms of pounds and the cube of the height. The smaller the index the smaller the relative volume. Chart K, p. 538, shows clearly that there is a close correlation between the decrease in the relative volume of the head and the increase in the relative volume of the lower extremities, on the one hand, and decrease in height-weight index on the other. In relative volume the trunk and upper extremities change comparatively little. In the latter part of adolescence and in maturity in the male, however, as the height-weight index increases the relative volume of the trunk increases. In the female the thighs appear to be about equally affected with the trunk in relative increase in volume. Data on this subject, however, are scanty.

If we assume that the volume of the body represents weight in pounds multiplied by 27 cubic inches and determine the ratio between the volume of the body and

the cube of the height we get a figure which also expresses the ratio of "average" cross-section of the body to square of height, since the height in inches is a diameter common to the two volumes compared. Thus, if  $v$  = volume of body,  $h$  = height,  $R$  = ratio of volume to cube of height,  $r$  = ratio of cross-section to square of height, then

$$R = v : h^3 = \frac{v}{h^3} : \frac{h^3}{h^3} = \frac{v}{h^3} : 1 = \frac{v}{h^3} : 1$$

The square root of this ratio gives what may be termed the ratio of "average" transverse diameter to height, thus:

$$\sqrt{\frac{v}{h^3}} : \sqrt{1} = \sqrt{\frac{v}{h^3}} : 1$$

In use of the metric system we get similar ratios if we calculate volume from weight in grams and specific gravity at 1.02516.

Instead of using the ratio between height and the diameter of an estimated volume we may make direct use of the height-weight ratio or of the height-weight index of build to obtain a mathematical expression of relative transverse diameter. The ratio of weight in pounds to cube of height in inches has the same value as the ratio of "average" cross-section of the body to square of height, as the following equation shows:

$$W : H^3 = \frac{W}{H^3} : \frac{H^3}{H^3} = \frac{W}{H^3} : 1$$

The square root of this ratio expresses a ratio of transverse diameter to height.

Assuming that a pound equals 27 cubic inches, this ratio would be  $\frac{1}{\sqrt{27}} = \frac{1}{5.1962}$  of the cubic-inch ratio described above. Thus, for the infant shown in figure 1 the square root of the height-weight ratio 0.0009178 is 0.030296, while the ratio of the square root of the cross-section in inches to the stature is 0.15742, which is 5.1962 times as great.

The height-weight index we have used is equal to the height-weight ratio  $\times 1000$ . Since this is equivalent to the ratio between weight in pounds and a cube of the tenth of the height in inches, it is necessary to divide this index by 10 or to multiply the height-weight ratio by 100 in order to get an equivalent index to express the relation between average cross-section and square of height. Index 0.918 thus becomes 0.0918 if used to express the transverse section ratio. We may call this the *transverse section index*. The square root of this index gives us a transverse diameter index which has 10 times the value of the transverse-diameter height ratio described above. Thus the ratio of the square root of the transverse section of volume estimated in inches from weight in the infant shown in figure 1 is 0.15742. In terms of the square root of the height-weight ratio it is 0.030296. The transverse-diameter-height index is 0.30296. This last is the more practical for ordinary use in dealing with inch-pound units. It is equal to about twice the square root of the transverse section estimated in inches and hence is equivalent to the long diameter of a cross-section 4 times as broad as thick. A curve based on this diameter ratio, the square root of the tenth part of the height-weight index, enables us to compare the curves of the ratios of linear measurements to stature with one expressing the ratio of a theoretical transverse diameter to stature. We have accordingly plotted such a curve "X" in charts I and J.

In chart I the most striking feature of this curve, illustrating the ratio of the "average" transverse diameter of the body to stature, is the fact that it is almost a mirror picture of the curve of the sitting-height, labeled "crotch" in the chart. Since these two curves have been plotted from entirely different data and from different standpoints it is of interest to see how much alike they are. The part of both curves up to the 30-inch line is somewhat artificial, since we are lacking in abundance of data for the first year of development. The beginnings of both curves at the 20-inch line are, however, based on a fair amount of data. Beyond the 30-inch line both curves are based on a relatively large amount of data. They

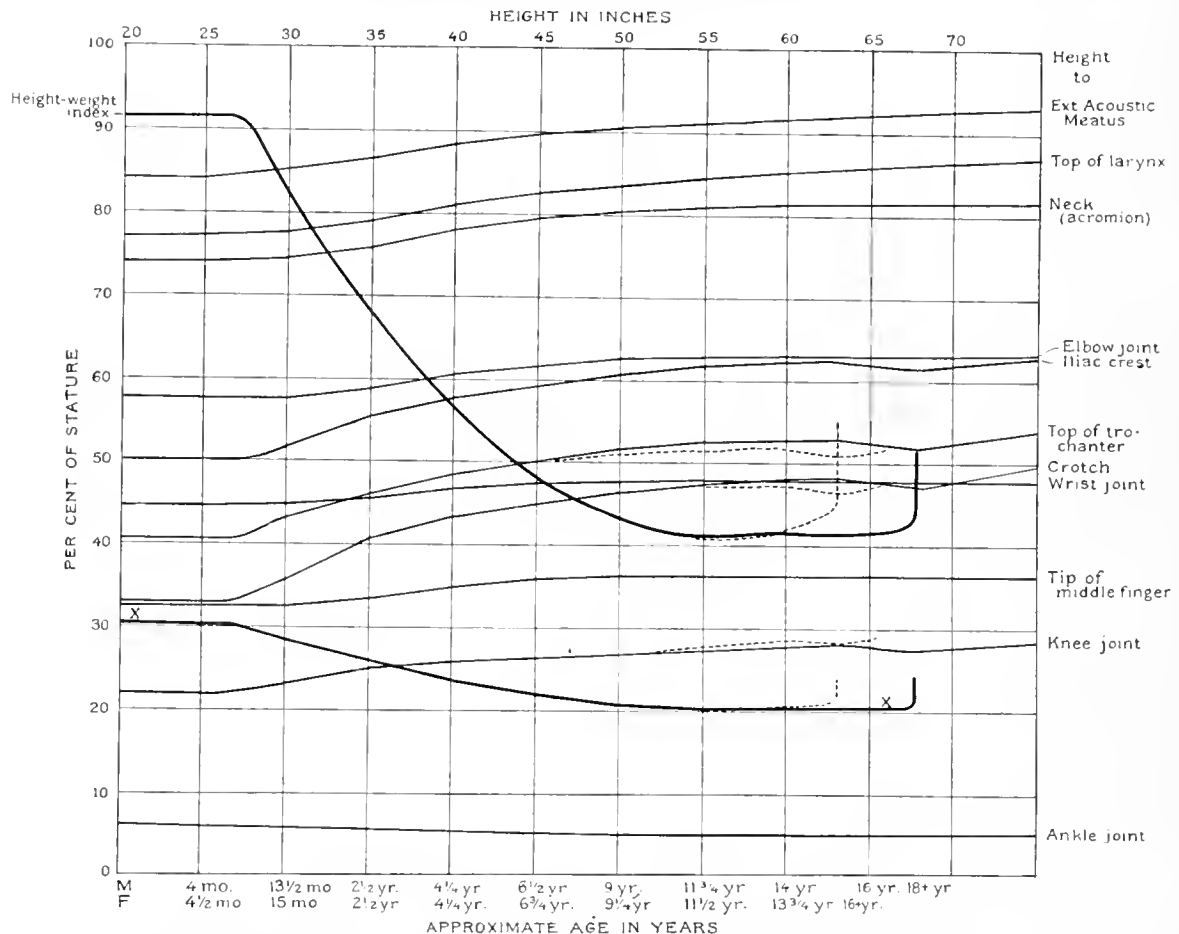


CHART I.—Height relative to stature of the level of the structures named at the right. Each level is shown by a curve extending from the left-hand to right-hand side. Horizontal lines indicate percentage of stature as shown at the left. Vertical lines indicate stature in inches as shown at the top. The approximate age at which the average healthy American reaches a given stature is shown at the bottom. The curved heavy line labeled "height-weight index" at the left represents the height-weight-index growth-curve. The heavy line marked by an X at each end represents the curve of the square root of a tenth of the height-weight index of growth. The dotted lines in the chart indicate the divergence of the relative proportions of the female from those of the male. The data on which the curves are based are given in table K.

show conclusively that the average transverse diameter of the body is in inverse proportion to the length of the free lower extremities. The ratio of the sitting-height, or of the length of the free lower extremities to stature and the height-weight index, are the two most valuable simple means we have of estimating the proportions of the body. They serve as a check on one another and should be made a matter of routine recording in the study of anatomical data.

In chart J two curves marked X, one on each side of the center, likewise represent the square root of a tenth of the height-weight index. Each curve is plotted at a distance from the mid-line equal to one-half of this "average" transverse diameter. On comparing this theoretical diameter with the curves of the various

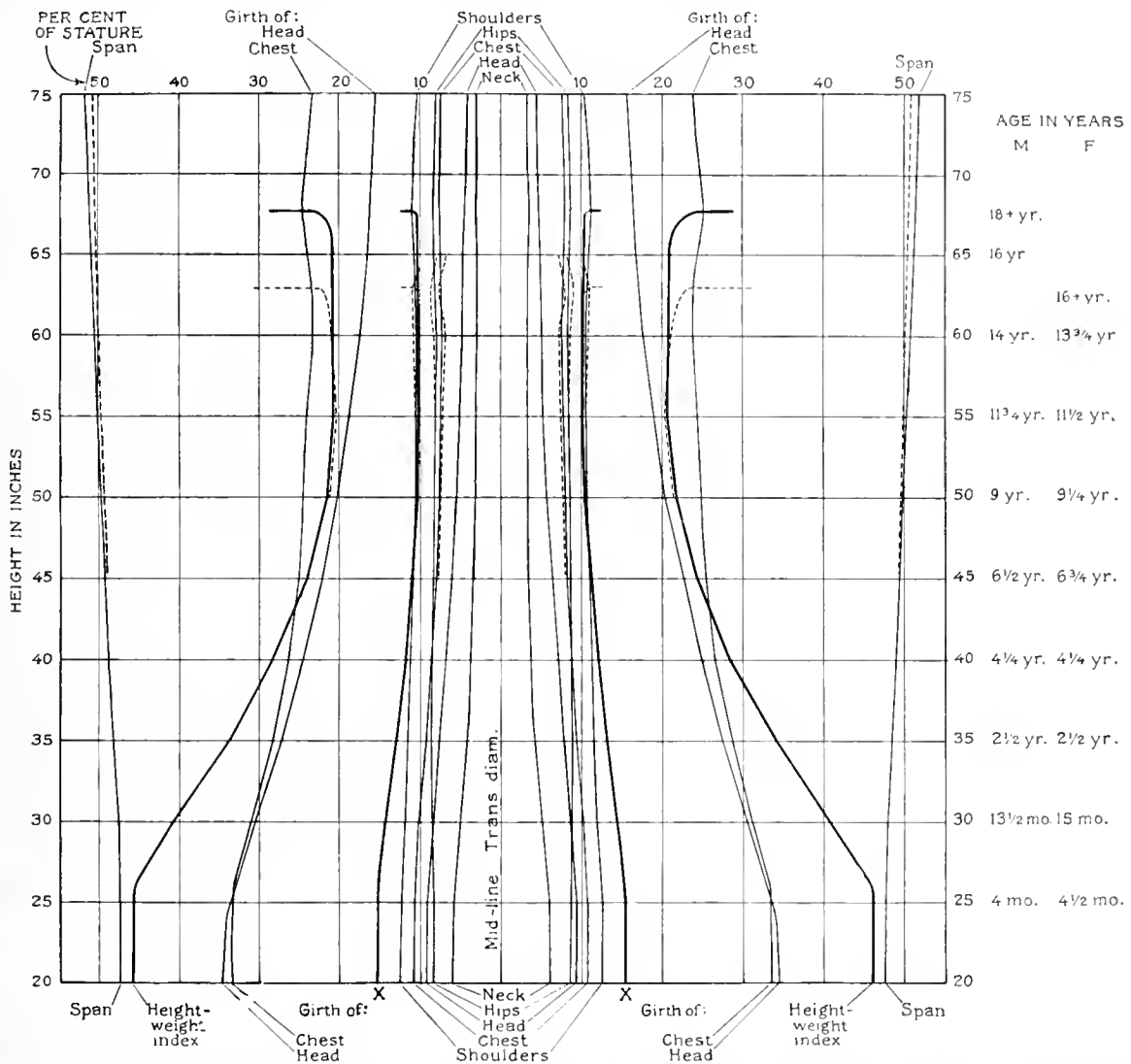


CHART J.—Length relative to stature of the span, of the girths of the head and chest, and of the width of the shoulders, hips, chest, head, and neck. The vertical line at the center represents the mid-sagittal plane. The vertical lines on each side of this represent distance from the mid-sagittal plane expressed in terms of percentage of stature as shown at the top. The horizontal lines represent height in inches as indicated at the left. At the right is the approximate age in years at which the average healthy American reaches a given stature. The light curved lines show the relative distance from the mid-sagittal plane of the ends of the transverse diameters given and of one-half the length of the chest-girth, of the head-girth, and of the span. The curve of the height-weight-index growth-curve and the curve of the square root of a tenth of this index are shown by heavy lines. The latter is marked by an X. Female curves, where they differ markedly from that of the male, are shown by dotted lines. The data on which the chart is based are given in table K.

actual transverse diameters, such as those of the shoulder, chest, head, hips, and neck, it may be seen to follow a similar course and to represent in a satisfactory manner the whole group of transverse diameters there shown. All of these transverse diameters converge toward the mid-line from infancy until the period preceding

puberty, but during adolescence diverge again and continue to diverge during maturity. The region of divergence centers at the height of full stature. The region of divergence in a group of short individuals is lower than that shown in the charts. The region of divergence in a group of tall individuals is higher and less marked. The girths show the same characteristics as the transverse diameters but to a greater degree. The girth of the head, however, shows little evidence of divergence at full stature. Pfitzner (1899) describes a continued increase in relative head-girth up to old age.

When using the metric-system units the simplest method of estimating average transverse section and average transverse diameter ratios is to use the metric height-weight index of build for the former and its square root for the latter. Since the metric height-weight index in reference to volume assumes a specific gravity of 1000 for a gram of tissue the ratio of cross-section to square of the stature thus estimated is approximately 2.5 per cent larger than when a specific gravity of 1.0252 is assumed, as we have in dealing with pounds and inches. The ratio of the average transverse diameter to stature is correspondingly increased 1.24 per cent. In comparison with the indices based on the inch-pound index of build, the metric transverse section ratio is 0.2768 as great, the transverse diameter index is  $\sqrt{0.2768}$  or 0.526 as great. The latter index is therefore approximately one-half as large as that based on the inch-pound height-weight index.

In table L we have estimated this centimeter-gram average transverse diameter ratio for each of the groups of individuals studied in order to facilitate a comparison between the ratios which it expresses and ratios of linear measurements to stature.

From Hitecock (1900) we have data on college students graded according to stature. The proportions for three such groups (*a*) 160 cm., (*b*) 175 cm. and (*c*) 183 cm. are given in table L. The average transverse diameter ratio of group (*a*) is 0.11472, of group (*b*) 0.10949, of group (*c*) 0.10559. There is thus a progressive decrease in average relative transverse diameter from the shortest to the tallest. In the tallest it is 7.95 per cent less than in the shortest.

Of the relative heights, the only one to show a progressive change in the same direction is sitting-height, which is 3.75 per cent less in group (*c*) than in group (*a*). The other heights, especially the height of the knee, show an increase. On the other hand the breadths of the head, neck, shoulders, and hips measured at the trochanters show a decrease of somewhat the same order of magnitude as the average transverse diameter. The width of the head shows the greatest decrease, that of the hips the least. Similar differences may be seen in the relative girths. The girth of the head here also shows the greatest decrease, but the decrease in girth of the hips at the trochanters is larger than that in breadth of hips and is of the same order of magnitude as that of the average transverse diameter. Anne L. Barr (Clapp) (1903) has furnished similar data for Nebraska college girls. Relative measurements of groups of stature (*a*) 150 cm., (*b*) 160 cm., and (*c*) 173 cm. are given in table L. Here also we find a decrease in relative average transverse diameter from 0.11662 in group (*a*) to 0.10719 in group (*c*), or 8.09 per cent. Sitting-height, breadths, and depths likewise show a decrease, but the girths (with the exception of those of the head, neck, and chest) are either essentially similar in the

three groups or show an increase. It is probable that some error has entered into the preparation of the tables for girths by this investigator, since the data on girths do not harmonize with the other data.

Hitchcock has furnished data on measurements of college students grouped according to age. If we compare subgroups of similar stature at the ages of 16 and 25 years (table L) we find that the average transverse diameter of the older group (*a*) is 3.59 per cent greater than that in the younger group (*b*). Corresponding with this we find an increase in relative sitting-height and in all breadths and girths with the exception of the breadth of the head, which is smaller in the older group.

TABLE 16.—Percentages of superiority and inferiority in relative measurements in a fat woman compared with college girls, stature 150 centimeters.

<i>Superiority:</i>		<i>Superiority—continued:</i>	
<i>Girths</i>	<i>p. ct.</i>	<i>Heights</i>	<i>p. ct.</i>
Head.....	6.1	Suprasternal notch.....	2.1
Neck.....	16.2	Sitting-height.....	9.72
Chest.....	36.1		
Abdomen.....	88.8	<i>Inferiority:</i>	
Hips at trochanters.....	43.0	Height of perineum.....	11.6
Arms.....	40.7	Height of pubic crest....	5.9
Forearms.....	33.3	Length of foot.....	2.0
Thighs.....	19.0		
Knees.....	26.2		
Calf.....	29.2		

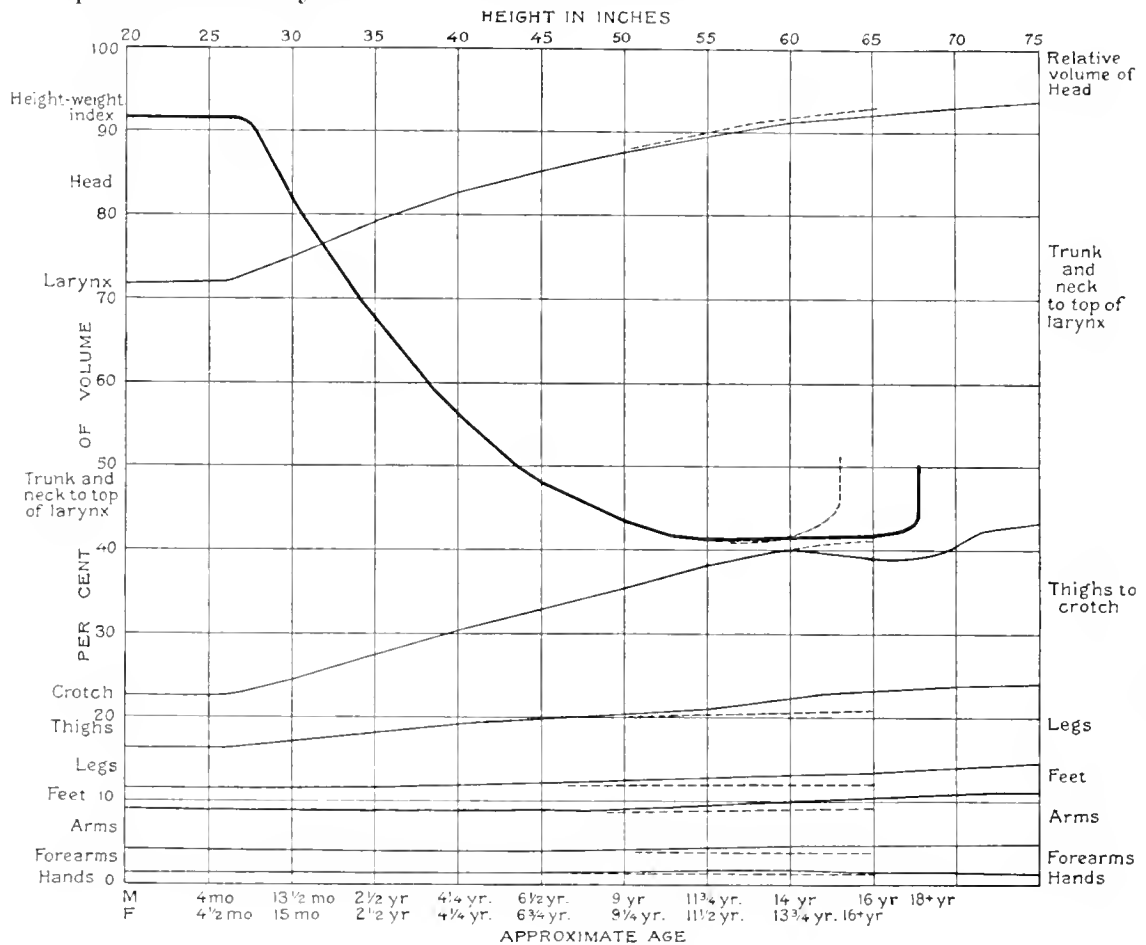
Du Bois and Du Bois (1915) have given linear measurements for an unusually fat woman, Mrs. McK., 149.7 cm. tall, weighing 93 kg. She thus has a metric height-weight index of build of 0.02772, a figure seldom found except in infants. The square root of this index is 0.1665. For the 150 cm. group of women in table L the corresponding figure is 0.1166. The average transverse diameter of Mrs. McK. is therefore 42.8 per cent greater than that of the Nebraska college girls of the same stature. It may be of interest to compare the percentage of difference in some of the relative measurements of the college girls and those of Mrs. McK. with the percentage of difference in average transverse diameter (table 16).

From these figures it may be seen that the trunk of the fat woman is relatively lengthened by increase in height of the super-sternal notch and decrease in height of the pubic crest and of the perineum. Allowance, however, should be made for the fact that the DuBois measurements are made in the recumbent position, the Nebraska measurements in the standing position. In the Nebraska data "sitting height" is given, in the DuBois data "height of perineum." It is here assumed, for the sake of making comparisons, that stature less sitting-height equals height to perineum. The foot appears to be slightly below average relative length. The inferior extremities are below average length. The superior extremities, not included in the table given above, are 48 per cent of the stature in length, which is above the normal.

The girths are all increased. Of those tabulated, that of the head is least increased. The greatest increase is in the girth of abdomen, 88.8 per cent—over twice that of the average transverse diameter, 42.8 per cent.

## CONCLUSIONS.

Chart K illustrates the changes which take place in the height-weight index of build during growth compared with the corresponding changes in volume of the chief parts of the body.



For description see p. 493.

CHART K.--Relative volume of the head to the level of the top of the larynx, of the trunk from here to the crotch, of the inferior extremities with their principal subdivisions, and of the superior extremities with their subdivisions. Light curved lines divide the volume of one part from that of another. The vertical lines represent stature in inches as shown at the top. The horizontal lines indicate percentage of total volume as shown at the left. The approximate age in years at which a given stature is reached is at the bottom. The typical height-weight-index growth-curve is shown by heavy line. Female curves, where they differ markedly from those of the male, are shown by dotted lines. The data on which this chart is based are given in table K.

In the use of the height-weight index of build as a method of estimating and recording the proportions of the body the following facts should be kept in mind:

1. The height-weight index is altered by the changes in external form which characterize post-natal morphogenesis in all individuals, by "physiological age."
2. The height-weight index is influenced by sexual peculiarities of structure.
3. The height-weight index is influenced by inherited individual or racial peculiarities of structure which may manifest themselves at any period of the life-cycle.
4. The height-weight index is influenced by peculiarities of structure due to habits of living or to environment.



1. The chief changes in the height-weight index associated with alterations in the proportions of the body during post-natal morphogenesis have been discussed above. They may be briefly summarized as follows: (a) First half-year after birth, *infancy*, rapid growth but relatively slight change in bodily proportions. (b) From this period to puberty, *childhood*, growth of limbs rapid, head slow, trunk intermediate, height-weight index greatly reduced. (c) Puberty to maturity, *adolescence*, growth of trunk and limbs at first about equal in the male, followed by a relatively more rapid growth of trunk. Growth in thickness of trunk continues longer than growth in length. During the first part of this period the index changes little, during the latter part it is increased. (d) Period of relatively stable proportions, *maturity*. Some increase in thickness of muscles during the early part of this period and of fat in the latter part usually causes a gradual increase in height-weight index during maturity. (e) Period of decline, *old age*. Loss of fat and musculature, decrease in stature and in relative length of spine. Decrease in index.

2. Sexual peculiarities are relatively small preceding puberty, although at the same age girls are usually somewhat smaller than boys in stature and build and have a slightly smaller index until the period immediately preceding puberty, when girls usually exceed boys in height, weight, and index. In girls at the time of puberty the trunk is elongated in the lumbar region and the pelvis is enlarged, while the lower extremities cease to grow proportionately with the trunk. Growth in stature ceases earlier than in boys and increase in width of trunk, except in the pelvic region, is less marked than in boys during the latter part of adolescence. Women have relatively bulkier thighs and calves than men; compared with men of the same height women usually have slightly longer lower extremities. The height-weight index is increased during the period of relative lengthening of the trunk and increase in size of the pelvic region and thighs. Increase in index due to increase in width of skeleton and musculature during adolescence is much less marked in women than in men, but increase in index due to adiposity is apt to be more marked, especially in the latter part of the period of maturity.

3. There have been numerous attempts to classify variations in bodily proportions into various types. Bean (1912) has endeavored to subdivide the human species into three main subdivisions from the standpoint of proportions of the body, the hypo-onto-morph, the meso-onto-morph, and the hyper-onto-morph. In the first, maturity comes on relatively early in the life-cycle, so that to a considerable extent the proportions of the child are preserved in the adult, large head, small face, long trunk, short lower limbs. According to Bean, it is a type characteristic of Asiatics and Filipinos. To some extent the data of Bobbitt (1909) appear to support Bean's views. On the other hand the marked increase in relative sitting-height, which takes place in these races after puberty, suggests an extension of the adolescent lengthening of the trunk at the expense of the pre-adolescent lengthening of limbs. Since the lower limbs in this type are relatively short, the height-index is relatively high in well-nourished individuals. The meso-onto-morph (according to Bean's descriptions) appears to come to maturity at a period corresponding to late childhood or early adolescence, a time when the limbs are

long, the trunk short and relatively slender. Feet and hands are relatively large, the face is large, compared with Europeans. According to Bean this type is characteristic of the negro, or at least of the majority of negroes. Negroes have a small pelvis (Manouvrier). In this type the height-weight index is relatively small. The hyper-onto-morph comes to maturity at a relatively later period than the other types. The period of lengthening of lower extremities is more prolonged than in the "Asiatic," the period of trunk lengthening more prolonged than in the "negro." It is the type the morphogenesis of which we have outlined in this paper. We have seen in this type that growth in the latter part of adolescence takes place chiefly in the trunk, so that while stature is increased the length of limbs compared to the trunk is decreased. The upper part of the trunk is especially affected, so that the distance from umbilicus to pubis is short compared with the other types (Bean). The distance umbilicus-to-pubis is relatively shorter in the French at 23.5 than at 13.5 (Godin, 1910). The trunk is also thickened. In this type, therefore, the height-weight index is relatively large, in fact larger than in the other two types, since the trunk is broad as compared with the hypo-onto-morph. The European races are essentially hyper-onto-morphic. The work of Bean is suggestive, but requires a large amount of careful statistical work for confirmation. According to the data given by Martin (1914) the Schikotan Ainos have the greatest relative sitting-height (males 54.8, females 54.6), the Australians the smallest (males 46.5, females 48.4).

Manouvrier (1902) has suggested that in the study of bodily proportions the individuals of the group studied be subdivided into three subgroups according to the relative length of the lower extremities, the short-legged or brachyskèles, the moderate-legged or mesatoskèles, and the long-legged or macroskèles. The mean height-weight index of the first subgroup is high as compared with that of the others, that of the last subgroup is low. Manouvrier's studies relate mainly to the French, hyper-meso-morphic from Bean's standpoint. In the macroskèles during adolescence we have relatively great growth of limbs as compared with the trunk, and relatively great growth in length of trunk as compared with width. In the brachyskèles we have relatively great growth of trunk as compared with the limbs, and usually of growth in thickness of trunks and limbs as compared with length. While all three subgroups may be found in individuals of any given stature, there are more brachyskèles among short individuals, more macroskèles among tall individuals. There is great variation in relative length of limbs in individuals of any given stature, excellently illustrated by Manouvrier (1902, figs. 1 and 2). The upper and lower extremities usually vary in the same direction, but in long-legged individuals the upper extremities are relatively less long than the lower extremities, and in short-legged individuals the upper extremities, while short compared with the trunk, are long compared with the lower extremities. In the typical brachyskèle, standing, the wrist reaches not much more than to the symphysis pubis, in the macroskèle to the perineum or below. In the brachyskèle the elbow does not reach the iliac crest, in the macroskèle it may.

While the short-legged individuals are usually stockier than long-legged individuals, this is not always the case. For broad or stocky build Manouvrier has offered the term "mégasomie," for slender build the term "microsomie," for the process leading to the former condition the term "euryplastie," for that leading to the latter condition "macroplastie." He has offered interesting suggestions as to the physiological conditions underlying these processes. Women, while short-legged (brachyskèlic), are of slender frame (microsomic). Godin (1910) has shown that short-legged individuals as a rule first begin to manifest marked relative thickening of the trunk and limbs (euryplastie) in the latter part of adolescence.

W. W. Mills (1917) has given an interesting and beautifully illustrated description of the differences in the topographical anatomy of the thorax and abdomen in those of stocky as compared with those of slender trunk. From this point of view he subdivides individuals into six classes, the hypersthenic (very stocky), hypersthenic-sthenic, sthenic, sthenic-hyposthenic, and hyposthenic (very long slender trunks).

4. Manouvrier has pointed out that physiological conditions may greatly modify the relative proportions of the body. Muscular work during childhood and adolescence tends to decrease stature (especially length of lower extremities through pressure on epiphyses) and increase stockiness of build. Sedentary life tends to promote length of lower extremities and slenderness of skeletal framework and musculature. In the former case we have increase of the height-weight index of build, in the latter a decrease. This last, however, may be modified by a relative increase of fat, which in turn increases height-weight index.

From this summary of the chief factors influencing height-weight index of build it may be seen that our judgment of build from height-weight index is greatly helped if we have some means of estimating relative length of the lower extremities and relative adiposity. Relative length of limbs may be most simply estimated from sitting-height as compared with stature. Relative adiposity may be most simply estimated from the relative circumference of the abdomen, or better from its relative antero-posterior diameter, since adiposity makes itself most clearly manifest here.

# APPENDIX I.

## TABLES A TO L.

The chief references to these tables are as follows: Table A, pp. 504, 516, 517; table B, pp. 488, 493 to 495; table C, p. 494; table D, pp. 501 to 517; table E, pp. 500 to 518; table F, pp. 501 to 516; table G, p. 486; tables H, I, J, pp. 521, 525 to 528; table K, pp. 493, 504 to 517; table L, pp. 507 to 519, 536 to 537.

TABLE A.—*Ratios of chest girth and of head girth to stature in infants and young children, based on data from Schmid-Monnard, 1892.*

Age, months.	Boys, Frankfurt on the Main.					Girls, Frankfurt on the Main.				
	823 individuals.				607 indiv.	736 individuals.			724 indiv.	731 indiv.
	Weight, grams.	Stature, centi- meters.	Index of build, in.-lb.	Relative chest- girth.	Relative head- girth.	Weight, grams.	Stature, centi- meters.	Index of build, in.-lb.	Relative chest- girth.	Relative head- girth.
1	3451	50.6	0.962	.628	.688	3219	50.1	0.926	.627	.671
2	4108	54.1	.938	.647	.691	4002	53.8	.929	.641	.673
3	4840	55.6	1.017	.604	.680	4792	57.5	.911	.630	.649
4	5670	59.9	.953	.651	.671	5409	59.3	.937	.632	.659
5	5868	60.5	.957	.623	.681	5866	61.0	.934	.636	.656
6	6802	63.0	.983	.654	.671	6426	62.2	.968	.627	.661
7	7017	64.4	.949	.624	.665	6855	64.0	.944	.622	.656
8	7152	66.1	.895	.640	.657	6936	64.9	.917	.613	.658
9	7579	67.4	.894	.616	.660	7396	66.9	.892	.604	.646
10	8312	65.9	1.049	.640	.675	7527	67.0	.904	.612	.655
11	8412	69.6	.901	.612	.642	7588	67.0	.912	.612	.655
12	8588	71.0	.867	.609	.644	7756	68.1	.886	.604	.652
13	8479	70.7	.867	.608	.642	8277	71.8	.808	.589	.624
14	8897	72.2	.854	.605	.640	8350	70.9	.847	.597	.636
15	8825	73.0	.820	.600	.633	8200	70.5	.845	.600	.641
16	9414	74.1	.836	.594	.630	8807	72.5	.835	.597	.633
17	9810	76.0	.808	.592	.619	9164	73.8	.824	.590	.627
18	9650	74.6	.840	.603	.629	9219	74.1	.818	.596	.613
19	9818	76.1	.805	.594	.620	9247	73.8	.831	.598	.623
20	9973	77.5	.774	.595	.605	9084	74.6	.791	.583	.613
21	9911	75.7	.826	.593	.616	9261	75.2	.785	.576	.612
22	10334	78.2	.781	.581	.618	9887	77.7	.762	.582	.596
23	10229	78.1	.776	.576	.613	9700	77.0	.768	.586	.603
24	10547	78.8	.779	.577	.609	10106	79.5	.727	.572	.585
25	10542	80.0	.741	.586	.598	10058	79.2	.732	.568	.587
26	11133	81.6	.740	.577	.596	10336	80.4	.719	.571	.583
27	11100	80.0	.783	.588	.606	10508	80.0	.741	.571	.584
28	11000	82.0	.720	.561	.600	10150	80.0	.716	.560	.589
29	11150	82.5	.718	.561	.586	11100	83.5	.689	.552	.571
30	11407	83.7	.703	.563	.584	10829	83.4	.675	.565	.561

TABLE B.—*Ratios of linear measurements to stature and of volumetric measurements to volume of body in 13 individuals studied by Meeh (1895) and in two additional children.*

Designation.	Sex.	Age, years.	Stature, centi- meters.	Weight, kilo- grams.	Index of build in.-lb.	Linear ratios.									
						Height, tro- chanter.	Tro- chanter to knee.	Knee to ankle.	Ankle to sole.	Upper extrem- ities.	Arm.	Fore- arm.	Hand.	Girth of head.	Girth of chest.
Meeh No. I.....	*Male.	0	55.0	3.956	0.860	0.418	0.200	0.164	0.055	0.418	0.173	0.127	0.118	0.673	0.600
Meeh No. III....	*Fem.	0	50.0	2.840	.820	.430	.210	.160	.060	.400	.160	.110	.130	.650	.600
Meeh No. IV....	*Male.	1.83	77.0	6.834	.542	.410	.190	.162	.058	.402	.162	.130	.110	.610	.558
C. R. B. No. 1....	*Male.	2.10	83.8	7.28	.447	.400	.180	.167	.053	.401	.167	.121	.114	.606	.545
C. R. B. No. 2....	Male.	6.20	118.1	20.86	.459	.499	.230	.215	.054	.436	.183	.140	.113	.445	.505
Meeh No. 8.....	Male.	11.85	138.5	30.47	.415	.524	.253	.227	.043	.444	.177	.152	.116	.372	.455
Meeh No. 7.....	Male.	13.17	137.5	28.47	.396	.545	.255	.255	.036	.429	.174	.153	.102	.383	.473
Meeh No. 6.....	Male.	17.09	161.0	50.77	.440	.534	.242	.244	.047	.447	.186	.146	.115	.348	.463
Meeh No. 5.....	Male.	22.06	162.0	54.70	.465	.531	.262	.231	.037	.466	.189	.157	.114	.346	.481
Meeh No. 2.....	Male.	27.00	171.0	59.85	.433	.523	.257	.216	.049	.426	.175	.146	.105	.348	.520
Meeh No. 4.....	Male.	31.21	154.5	62.40	.610	.505	.239	.223	.042	.424	.165	.149	.110	.375	.605
Meeh No. 1.....	Male.	42.50	169.4	68.30	.508	.525	.248	.236	.041	.460	.189	.159	.112	.325	.566
Meeh No. 3.....	Male.	55.75	161.0	47.80	.414	.528	.273	.217	.037	.444	.177	.158	.109	.323	.512
Meeh No. 9.....	Fem.	16.25	154.5	54.55	.534	.531	.249	.233	.049	.434	.175	.142	.116	.350	.569
Meeh No. 10....	Fem.	22.21	156.0	60.23	.573	.526	.256	.226	.045	.426	.183	.141	.102	.337	.564

The maximum and minimum ratios of several measurements are in italics.

\*Cadaver.

TABLE B.—*Ratios of linear measurements to stature and of volumetric measurements to volume of body in 13 individuals studied by Meeh (1895) and in two additional children—continued.*

Designation.	Sex.	Stature, centi- meters	Volume, liters.	Specific gravity.	Volumetric ratios.										
					Head.	Neck.	Trunk.	Lower extremi- ties.	Thigh.	Leg.	Foot.	Upper extremi- ties.	Arm.	Fore- arm.	Hand.
Meeh No. I.....	*Male.	55.0	3.757	1.053	0.2667	0.0492	0.4437	0.1523	0.0418	0.0228	0.0116	0.0881	0.0242	0.0138	0.0061
Meeh No. III....	*Fem.	50.0	2.728	1.041	.2580	.1008	.4176	.1334	.0284	.0255	.0128	.0902	.0225	.0145	.0081
Meeh No. IV.....	*Male.	77.0	6.626	1.031	.2649	.0347	.4798	.1456	.0330	.0260	.0127	.0750	.0186	.0122	.0067
C. R. B. No. 1....	*Male.	83.8	12.60	.....	.2200	.....	.5150	.1750	.0415	.0310	.0150	.0900	.0230	.0150	.0070
C. R. B. No. 2....	Male.	118.1	20.35	.....	.1523	.....	.5103	.2480	.0658	.0415	.0167	.0894	.0233	.0147	.0066
Meeh No. 8.....	Male.	138.5	28.46	1.071	.1075	.0322	.4381	.3220	.0875	.0525	.0210	.1003	.0241	.0160	.0100
Meeh No. 7.....	Male.	137.5	30.10	.946	.0887	.0249	.5139	.2817	.0803	.0415	.0191	.0907	.0232	.0130	.0091
Meeh No. 6.....	Male.	161.0	50.61	1.003	.0721	.0239	.5083	.2900	.0786	.0486	.0178	.1056	.0303	.0154	.0072
Meeh No. 5.....	Male.	162.0	52.87	1.034	.0792	.0304	.4696	.3006	.0845	.0492	.0167	.1201	.0342	.0178	.0081
Meeh No. 2.....	Male.	171.0	61.66	.971	.0653	.0281	.5301	.2882	.0861	.0438	.0141	.0882	.0246	.0137	.0058
Meeh No. 4.....	Male.	154.5	64.04	.974	.0634	.0285	.5656	.2403	.0706	.0362	.0134	.1022	.0291	.0161	.0059
Meeh No. 1.....	Male.	169.4	67.22	1.061	.0735	.0412	.5030	.2805	.0855	.0408	.0138	.1017	.0307	.0144	.0057
Meeh No. 3.....	Male.	161.0	50.26	.951	.1002	.0205	.5235	.2632	.0800	.0358	.0158	.0925	.0256	.0143	.0064
Meeh No. 9.....	Fem.	154.5	54.21	1.006	.0695	.0181	.5000	.3174	.0987	.0473	.0127	.0951	.0278	.0136	.0061
Meeh No. 10....	Fem.	156.0	59.31	1.016	.0626	.0248	.4966	.3237	.1022	.0457	.0139	.0924	.0273	.0135	.0054

The maximum and minimum ratios of several measurements are in italics.

\*Cadaver.

TABLE C.—*Ratios of weights of parts of body to weight of whole body in two infants and one child studied by Meeh (1895), in two adults studied by Harless (1857), and in one adult studied by Braune and Fischer (1890).*

Designation.	Sex.	Age, years.	Weight, kilo-grams.	Ratio of weight of—									
				Head.	Trunk.	Upper extremities.	Arm.	Fore-arm.	Hand.	Lower extremities.	Thigh.	Leg.	Foot.
Meeh I.....	Male.	0	3.956	0.2666	0.5041	0.0942	0.0258	0.0147	0.0066	0.1350	0.0337	0.0225	0.0114
Meeh III.....	Fem.	0	2.840	.2709	.5018	.0915	.0228	.0149	.0081	.1358	.0291	.0259	.0129
Meeh IV.....	Male.	1.83	6.834	.2814	.4923	.0778	.0192	.0126	.0071	.1486	.0341	.0267	.0134
Harless, Graf.....	Male.	.....	63.97	.0712	.4628	.1179	.0324	.0181	.0084	.3482	.1120	.0438	.0183
Harless, Kefer.....	Male.	29.0	46.71	.0802	.4168	.1125	.0310	.0170	.0082	.3905	.1261	.0481	.0210
Braune and Fischer.....	Male.	.....	58.7	.0705	.4259	.1298	.0337	.0228	.0081	.3728	.1159	.0526	.0179

TABLE D.—*Ratios of linear measurements of several regions of the body to stature at various ages, based on data from Weissenberg (1911).*  
Stature=1000. M=Male. F=Female.

Age, years.	Stature in centimeters.		Trunk acromion to seat.		Head and neck.		Sitting-height.		Trochanter to sole.		Trochanter to level of seat.		Length in relation to stature.									
													Span.		Chest girth.		Width, shoulders.		Width, hips.		Wgt., K.	Index of build, males.
	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	em.-gm. in.-lb.
0	50.8	50.0	421	424	244	242	665	666	403	406	068	072	957	960	555	570	211	208	153	154	.....	.....
2	80.6	78.5	364	.....	248	.....	612	608	447	447	059	055	993	.....	600	.....	233	.....	175	.....	.....	.....
3	87.2	87.8	359	.....	236	.....	595	583	455	458	050	041	995	.....	580	.....	229	.....	177	.....	.....	.....
4	94.3	92.3	354	.....	227	.....	581	578	465	471	046	049	994	.....	557	.....	226	.....	171	.....	.....	.....
5	100.5	99.8	350	.....	221	.....	571	571	468	478	039	051	1000	.....	536	.....	223	.....	172	.....	16.18	0.0159 0.476
6	108.3	106.1	344	341	216	218	560	559	485	486	045	045	1002	987	521	509	223	218	170	169	19.17	.0151 .545
7	113.3	111.8	341	342	212	213	553	555	493	495	046	050	999	995	503	503	219	219	167	166	20.02	.0138 .499
8	117.1	116.7	339	339	210	207	549	546	498	502	047	048	1001	996	501	488	219	217	167	165	22.14	.0138 .498
9	123.2	122.9	340	334	199	203	539	537	502	508	041	045	976	1002	502	486	212	216	164	164	24.45	.0131 .472
10	126.5	128.6	337	341	199	195	536	536	507	509	043	045	1001	1000	495	479	216	214	164	164	25.69	.0127 .459
11	132.3	132.0	330	339	194	191	524	530	519	513	043	043	1006	1001	492	471	212	212	163	162	27.29	.0118 .426
12	137.5	137.9	325	338	194	191	519	529	525	518	044	047	1013	1011	474	480	210	212	161	164	30.75	.0118 .426
13	141.3	144.5	327	337	192	191	519	525	524	520	043	045	1013	1012	486	483	211	213	161	167	33.34	.0118 .426
14	146.0	149.2	326	338	188	188	514	527	529	521	046	048	1014	1016	490	485	210	212	162	169	37.89	.0122 .440
15	153.7	150.5	325	347	185	189	510	536	532	514	042	050	1025	1017	493	501	211	215	163	174	40.98	.0113 .408
16	158.9	152.0	328	341	187	189	515	536	529	515	044	051	1031	1034	489	511	213	216	162	176	46.34	.0116 .417
17	162.5	153.2	335	351	187	195	522	539	525	513	047	052	1041	1020	494	507	214	217	164	177	51.40	.0120 .433
18	162.7	154.6	340	354	185	188	525	537	522	512	047	049	1030	1025	506	516	214	218	166	177	53.98	.0125 .452
19	162.8	153.8	337	353	189	183	526	536	523	514	049	050	1039	1020	508	515	220	215	168	179	56.75	.0131 .473
20	164.4	153.9	338	356	187	184	525	540	521	510	046	050	1036	1022	506	523	218	218	167	179	56.60	.0127 .460
21 to 25	165.8	153.9	342	356	184	180	526	536	520	512	046	048	1039	1024	508	525	218	218	166	182	58.51	.0128 .462
26 to 30	164.7	153.5	344	356	185	182	529	538	520	513	049	051	1041	1022	527	525	220	220	168	182	61.69	.0138 .500
31 to 40	163.3	153.4	346	354	181	181	527	535	516	513	043	048	1042	1029	533	542	222	220	169	185	60.45	.0139 .502
41 to 50	163.4	153.3	346	357	185	179	531	536	517	513	048	049	1040	1033	539	560	223	224	171	188	62.92	.0144 .520
51 to 60	161.8	151.3	349	350	183	183	532	533	522	517	054	050	1052	1038	543	548	225	223	172	190	61.42	.0145 .524
61 to 75	163.0	147.9	345	347	181	182	526	529	520	517	046	046	1048	1044	532	560	222	227	177	193	.....	.....

Maximum stature and the maximum and minimum ratios of several measurements are in italics.

TABLE E.—*Ratio of linear measurements of various parts of the body to stature during growth based on data from Quetelet (1870).*

Age, years.	Stature, centimeters.		Weight, kilos.		Relative distance vertex to—											
					External acoustic meatus.		Termination of chin.		Clavicles.		Umbilicus.		Haunches.		Pubic crest.	
	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.
A, Birth	50.0	49.4	3.1	3.0	0.160	0.162	0.231	0.233	0.280	0.280	0.550	0.550	0.565	0.565	0.636	0.636
1	69.8	69.0	9.0	8.6	.150	.152	.226	.229	.256	.256	.525	.525	.535	.535	.610	.610
2	79.1	78.1	11.0	11.0	.145	.145	.219	.222	.239	.239	.506	.506	.514	.514	.590	.590
3	86.4	85.4	12.5	12.4	.137	.137	.211	.214	.228	.228	.489	.489	.497	.497	.575	.576
4	92.7	91.5	14.0	13.9	.130	.130	.203	.206	.218	.218	.475	.476	.484	.485	.563	.564
5	98.7	97.4	15.9	15.3	.123	.123	.195	.197	.210	.210	.463	.464	.472	.473	.552	.554
6	104.6	103.1	17.8	16.7	.117	.117	.188	.190	.204	.203	.452	.454	.461	.463	.542	.545
7	110.4	108.7	19.7	17.8	.111	.112	.180	.182	.198	.197	.442	.445	.452	.455	.533	.536
8	116.2	114.2	21.6	19.0	.106	.108	.173	.175	.194	.192	.433	.436	.443	.447	.525	.528
9	121.8	119.6	23.5	21.0	.102	.104	.167	.169	.190	.188	.426	.429	.436	.440	.518	.521
10	127.3	124.9	25.2	23.1	.098	.100	.161	.163	.187	.185	.419	.423	.430	.434	.513	.517
11	132.5	130.1	27.0	25.5	.095	.096	.156	.158	.184	.182	.415	.419	.425	.429	.508	.512
12	137.5	135.2	29.0	29.0	.092	.093	.152	.153	.181	.178	.410	.415	.420	.425	.504	.509
13	142.3	140.0	33.1	32.5	.090	.089	.149	.149	.179	.176	.407	.412	.416	.422	.500	.506
14	146.9	144.6	37.1	36.3	.088	.086	.147	.145	.177	.175	.403	.409	.414	.421	.498	.504
15	151.3	148.8	41.2	40.0	.086	.085	.145	.141	.176	.175	.400	.406	.413	.421	.496	.502
16	155.4	152.1	45.4	43.5	.084	.083	.143	.139	.175	.174	.398	.404	.412	.421	.495	.502
17	159.4	154.6	49.7	46.8	.082	.081	.141	.138	.174	.174	.397	.403	.412	.420	.494	.502
18	163.0	156.3	53.9	49.8	.080	.081	.139	.138	.173	.173	.396	.402	.412	.420	.493	.502
19	165.5	157.0	57.0	52.1	.079	.080	.138	.139	.172	.172	.396	.402	.412	.420	.493	.502
20	167.0	157.4	59.5	53.2	.078	.080	.138	.139	.171	.171	.396	.402	.412	.420	.492	.502
25	168.2	157.8	66.2	54.8	.078	.080	.137	.140	.171	.171	.396	.402	.412	.420	.492	.502
30	168.6	158.0	66.1	55.3	.078	.080	.136	.140	.171	.171	.397	.403	.412	.420	.493	.504
Z, 40	168.6	158.0	63.7	55.2	.078	.080	.136	.140	.171	.171	.398	.404	.412	.420	.493	.506
$\frac{Z}{A}$	3.37	3.19	20.5	18.4	0.48	0.49	0.59	0.61	0.61	0.61	0.72	0.73	0.73	0.74	0.77	0.80

Age, years.	Relative distance acromion to—						Relative distance sole to—								Length, foot.	
	Medius.		Wrist.		Elbow.		Crotch.		Trochanter.		Knee.		Ankle.			
	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.
A, Birth.	0.412	0.412	0.290	0.290	0.177	0.177	0.320	0.320	0.390	0.390	0.230	0.230	0.054	0.054	0.150	0.150
1	.416	.416	.296	.296	.180	.180	.345	.345	.410	.410	.240	.240	.052	.052	.153	.152
2	.419	.418	.301	.300	.182	.181	.364	.364	.427	.427	.247	.247	.051	.051	.155	.154
3	.422	.420	.305	.303	.184	.182	.380	.379	.441	.441	.251	.251	.050	.050	.157	.155
4	.425	.422	.310	.307	.185	.182	.395	.394	.454	.453	.255	.254	.050	.050	.158	.156
5	.427	.423	.312	.309	.186	.183	.409	.407	.465	.464	.259	.258	.050	.050	.159	.157
6	.429	.424	.315	.310	.187	.183	.422	.420	.473	.472	.262	.261	.050	.050	.160	.158
7	.430	.425	.317	.312	.187	.183	.433	.430	.481	.479	.266	.264	.050	.050	.160	.158
8	.433	.425	.320	.313	.188	.184	.443	.440	.488	.486	.268	.266	.049	.049	.160	.158
9	.435	.425	.322	.314	.188	.184	.452	.448	.495	.492	.271	.268	.049	.049	.161	.159
10	.437	.426	.324	.316	.189	.185	.460	.456	.500	.497	.274	.271	.049	.049	.161	.158
11	.439	.427	.326	.317	.190	.185	.466	.461	.505	.501	.276	.273	.049	.049	.161	.158
12	.440	.428	.327	.318	.190	.186	.470	.464	.509	.504	.278	.275	.049	.049	.161	.157
13	.442	.429	.329	.318	.191	.187	.474	.467	.513	.508	.280	.277	.048	.048	.161	.157
14	.444	.431	.331	.319	.192	.188	.477	.468	.515	.510	.282	.279	.048	.048	.162	.156
15	.446	.433	.333	.320	.193	.189	.478	.469	.517	.510	.283	.280	.048	.048	.162	.155
16	.448	.435	.335	.322	.194	.190	.479	.469	.518	.510	.284	.280	.048	.048	.162	.154
17	.450	.437	.337	.324	.194	.190	.480	.469	.519	.510	.284	.280	.048	.048	.161	.153
18	.451	.439	.338	.326	.195	.190	.480	.469	.520	.510	.284	.280	.049	.049	.160	.151
19	.453	.441	.340	.328	.195	.190	.480	.469	.520	.510	.284	.280	.049	.049	.159	.150
20	.454	.442	.341	.329	.196	.190	.480	.469	.520	.510	.284	.280	.049	.049	.158	.150
25	.455	.442	.342	.329	.197	.190	.480	.468	.520	.510	.284	.280	.050	.050	.157	.149
30	.455	.442	.342	.329	.198	.190	.479	.467	.520	.509	.283	.279	.050	.050	.157	.149
Z, 40	.455	.442	.342	.329	.198	.190	.478	.466	.520	.508	.283	.279	.050	.050	.157	.149
$\frac{Z}{A}$	1.10	1.07	1.19	1.14	1.12	1.07	1.50	1.46	1.33	1.30	1.23	1.21	0.93	0.93	1.05	1.00

TABLE E.—Ratio of linear measurements of various parts of the body to stature during growth based on data from Quetelet (1870)—continued.

Age, years.	Height-weight index of build.				Relative transverse diameters.											
	Centim.-gram, per cent.		Inch.-pound.		Head.		Neck.		Bi-acromial.		Bi-axillary.		Bi-ilio- spinal.		Bi-tro- chanteric.	
	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.
A, Birth.	2.48	2.49	0.896	0.899	0.200	0.202	0.092	0.092	0.245	0.245	0.195	0.195	0.159	0.159	0.210	0.210
1	2.65	2.62	.957	.946	.182	.183	.099	.099	.245	.245	.217	.217	.164	.164	.231	.231
2	2.22	2.31	.802	.834	.170	.171	.087	.087	.244	.244	.205	.205	.163	.163	.220	.220
3	1.94	1.99	.701	.718	.159	.160	.079	.079	.243	.243	.197	.197	.160	.160	.210	.210
4	1.76	1.82	.636	.657	.149	.150	.074	.075	.241	.241	.190	.190	.157	.157	.203	.203
5	1.65	1.65	.596	.596	.141	.142	.071	.072	.238	.238	.185	.184	.151	.154	.197	.197
6	1.55	1.52	.560	.549	.134	.135	.068	.070	.235	.235	.180	.179	.150	.150	.193	.192
7	1.46	1.39	.527	.502	.128	.129	.067	.069	.231	.231	.176	.174	.147	.146	.190	.188
8	1.38	1.28	.498	.462	.123	.124	.065	.068	.228	.227	.173	.170	.141	.143	.188	.185
9	1.30	1.35	.469	.487	.118	.118	.064	.068	.227	.223	.170	.166	.142	.140	.187	.183
10	1.22	1.19	.440	.429	.114	.114	.063	.067	.227	.220	.168	.163	.140	.138	.186	.181
11	1.16	1.16	.419	.419	.110	.109	.062	.067	.227	.217	.167	.160	.139	.136	.186	.180
12	1.12	1.17	.404	.422	.107	.106	.062	.067	.227	.215	.167	.158	.139	.136	.185	.180
13	1.15	1.15	.415	.415	.104	.102	.061	.068	.227	.214	.167	.156	.138	.137	.184	.182
14	1.17	1.20	.422	.433	.101	.099	.061	.068	.227	.213	.168	.154	.138	.138	.183	.185
15	1.11	1.21	.401	.437	.098	.097	.061	.069	.227	.213	.168	.153	.138	.139	.184	.189
16	1.21	1.24	.437	.448	.096	.095	.061	.070	.228	.214	.169	.154	.139	.140	.185	.194
17	1.22	1.24	.440	.448	.094	.094	.061	.071	.229	.215	.171	.157	.139	.141	.188	.198
18	1.25	1.30	.451	.469	.092	.094	.062	.072	.231	.217	.173	.160	.139	.142	.191	.202
19	1.23	1.35	.444	.487	.091	.093	.063	.073	.233	.219	.175	.163	.140	.144	.193	.201
20	1.28	1.36	.462	.491	.091	.093	.065	.074	.234	.220	.176	.165	.140	.146	.193	.206
25	1.39	1.39	.502	.502	.091	.093	.070	.076	.234	.220	.176	.168	.140	.149	.193	.207
30	1.38	1.39	.498	.502	.091	.093	.071	.077	.234	.220	.176	.170	.140	.150	.193	.208
Z, 40	1.33	1.40	.480	.505	.091	.093	.072	.078	.234	.220	.176	.172	.140	.150	.193	.208
$\frac{Z}{A}$	1.86	1.78	1.86	1.78	0.45	0.46	0.78	0.85	0.96	0.90	0.90	0.87	0.88	0.94	0.92	0.99

Age, years.	Relative girths.															
	Head.		Thorax.		Waist.		Pelvis.		Arm.		Thigh.		Knee.		Calf.	
	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.
A, Birth.	0.670	0.676	0.590	0.590	0.562	0.562	0.485	0.485	0.183	0.183	0.277	0.277	0.228	0.228	0.196	0.196
1	.631	.646	.630	.630	.665	.665	.655	.655	.192	.192	.339	.339	.265	.265	.237	.237
2	.595	.600	.585	.585	.630	.625	.632	.631	.170	.170	.318	.318	.243	.243	.219	.219
3	.563	.567	.560	.559	.591	.583	.598	.593	.159	.159	.307	.307	.232	.231	.211	.210
4	.535	.538	.543	.539	.560	.548	.570	.566	.150	.150	.299	.298	.225	.223	.206	.204
5	.510	.512	.530	.520	.533	.516	.549	.544	.144	.144	.292	.290	.219	.217	.203	.201
6	.486	.490	.520	.506	.512	.490	.531	.525	.139	.139	.286	.284	.215	.212	.200	.198
7	.465	.467	.511	.495	.495	.471	.518	.505	.135	.135	.281	.279	.212	.208	.198	.195
8	.447	.449	.504	.487	.481	.456	.504	.489	.132	.132	.277	.274	.209	.205	.196	.192
9	.430	.429	.499	.479	.470	.443	.493	.475	.130	.130	.275	.272	.207	.202	.195	.189
10	.415	.414	.495	.474	.460	.433	.485	.467	.128	.128	.273	.269	.207	.200	.194	.185
11	.402	.398	.492	.470	.451	.424	.479	.464	.127	.127	.271	.269	.206	.199	.193	.184
12	.389	.384	.491	.467	.447	.417	.474	.465	.126	.126	.270	.270	.206	.198	.192	.183
13	.378	.371	.490	.466	.445	.414	.472	.468	.126	.126	.270	.273	.205	.199	.191	.183
14	.368	.360	.490	.467	.444	.413	.471	.476	.127	.126	.270	.278	.205	.200	.190	.184
15	.361	.351	.491	.471	.443	.412	.471	.490	.130	.130	.271	.283	.205	.203	.190	.187
16	.355	.345	.493	.481	.442	.413	.472	.500	.135	.135	.274	.290	.205	.207	.191	.194
17	.350	.340	.497	.492	.442	.414	.472	.510	.138	.140	.277	.298	.206	.209	.193	.200
18	.345	.341	.504	.500	.443	.415	.473	.518	.141	.145	.279	.305	.206	.210	.198	.202
19	.341	.340	.511	.507	.443	.417	.473	.524	.144	.148	.282	.311	.206	.210	.202	.204
20	.338	.340	.518	.511	.444	.421	.473	.527	.147	.149	.284	.316	.206	.211	.203	.204
25	.336	.340	.525	.514	.444	.425	.473	.529	.148	.150	.287	.317	.206	.211	.204	.204
30	.335	.340	.528	.515	.444	.430	.473	.530	.150	.150	.287	.317	.206	.211	.204	.204
Z, 40	.335	.340	.528	.515	.444	.430	.473	.530	.152	.146	.287	.317	.206	.210	.204	.204
$\frac{Z}{A}$	0.50	0.50	0.90	0.88	0.79	0.77	0.97	1.10	0.83	0.80	1.04	1.14	0.91	0.92	1.04	1.04

TABLE F.—*Ratios of linear measurements of several regions of the body to the stature at various ages, based on data from Hastings (1902).*

Age, years.	Males.			Females.			Sitting-height.		Span of arms.		Breadth of chest.		Depth of chest.		Girth of head.	
	Height, cm.	Weight, kilos.	Index of build.	Height, cm.	Weight, kilos.	Index of build.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.
5	98.00	15.99	0.614	98.00	15.23	0.584	0.546	0.561	0.959	0.962	0.173	0.173	0.123	0.125	0.510	0.502
5	100.00	16.31	.590	100.00	16.02	.579	.577	.573	.993	.978	.175	.179	.124	.122	.510	.501
5	102.00	17.29	.588	102.00	16.84	.573	.577	.572	.986	.973	.175	.173	.122	.120	.501	.494
5	104.00	17.50	.563	104.00	17.27	.555	.566	.560	.972	.997	.172	.170	.119	.119	.490	.483
5	106.00	18.26	.554	106.00	18.22	.551	.563	.567	.972	.976	.171	.166	.117	.118	.484	.481
5	105.78	17.86	.546	105.38	17.32	.531	.561	.563	.969	.970	.171	.168	.117	.117	.484	.476
5	108.00	18.84	.540	108.00	18.86	.540	.558	.558	.969	.981	.169	.162	.114	.116	.475	.474
5	110.00	19.20	.521	110.00	18.71	.507	.565	.555	.975	.973	.166	.162	.112	.114	.473	.457
5	112.00	21.02	.541	112.00	20.74	.536	.559	.565	.981	1.001	.169	.166	.117	.113	.462	.453
6	110.67	19.37	.516	109.90	18.50	.506	.553	.558	.977	.976	.167	.161	.116	.112	.466	.459
7	115.69	21.30	.497	114.95	20.70	.490	.554	.555	.988	.975	.164	.160	.112	.110	.451	.443
8	121.31	23.14	.469	120.16	22.17	.461	.544	.550	.983	.977	.160	.157	.106	.105	.430	.424
9	125.86	25.07	.454	126.17	24.90	.448	.540	.532	.991	.974	.157	.153	.106	.104	.417	.411
10	130.95	27.85	.448	131.29	27.16	.431	.538	.529	.993	.983	.155	.151	.102	.100	.399	.396
11	134.90	29.86	.441	135.16	29.00	.424	.531	.527	1.005	.982	.155	.153	.100	.097	.392	.389
12	140.29	32.98	.432	142.03	33.06	.417	.525	.523	1.001	.992	.154	.150	.101	.097	.380	.373
13	145.09	35.60	.421	148.53	37.94	.416	.518	.521	1.006	.990	.152	.150	.099	.095	.369	.360
14	151.02	39.73	.417	153.17	42.92	.434	.517	.527	1.009	.999	.151	.150	.099	.095	.359	.353
15	158.18	46.95	.428	156.79	46.71	.434	.510	.531	1.014	1.001	.150	.152	.101	.096	.345	.345
16	163.73	52.90	.431	157.93	50.38	.462	.520	.536	1.027	1.002	.153	.154	.103	.101	.334	.348
17	169.98	56.82	.418	159.40	50.44	.450	.511	.521	1.028	1.006	.150	.154	.108	.105	.333	.346
18	171.07	59.25	.427	159.74	50.16	.444	.523	.521	1.038	1.006	.151	.156	.109	.104	.328	.345
19	171.81	61.71	.439	160.09	51.43	.453	.526	.527	1.034	1.013	.155	.156	.110	.104	.332	.344
20	163.00	54.59	.459	152.00	45.00	.464	.535	.536	1.028	1.020	.157	.157	.113	.105	.345	.360
20	166.00	59.14	.472	154.00	50.45	.500	.537	.532	1.054	1.019	.157	.159	.111	.105	.343	.358
20	169.00	59.32	.450	156.00	51.14	.487	.531	.532	1.046	1.019	.159	.157	.110	.105	.334	.354
20	172.00	60.45	.430	158.00	51.88	.475	.530	.523	1.045	1.019	.155	.157	.108	.108	.333	.348
20	172.22	61.09	.434	160.00	51.82	.457	.528	.532	1.021	1.027	.160	.157	.112	.107	.331	.344
20	175.00	63.68	.429	160.81	52.27	.454	.522	.530	1.043	1.020	.159	.156	.106	.106	.328	.343
20	178.00	65.18	.417	162.00	52.05	.441	.522	.524	1.037	1.018	.151	.157	.108	.105	.324	.342
20	181.00	66.93	.378	164.00	53.64	.438	.521	.517	1.028	1.012	.149	.153	.108	.104	.317	.336
20	184.00	74.77	.434	166.00	56.99	.450	.522	.527	1.035	1.021	.153	.154	.109	.103	.313	.337

At the ages of 5 and 20 years the ratios at several heights are given, and the mean stature for each age is in italics. The maximum or minimum intermediate ratios for several measurements are likewise in italics. The height-weight index of build is given on the inch-pound basis; weight, clothed without shoes.

TABLE G.—*Annual increase of stature during school years, after data from Boas (1912) and from Baldwin (1914).*

Year.	Boas.		Baldwin.							
	Males, growth.	Females, growth.	Males.				Females.			
			Short.		Tall.		Short.		Tall.	
			Growth.	Per cent.	Growth.	Per cent.	Growth.	Per cent.	Growth.	Per cent.
	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>		<i>mm.</i>		<i>mm.</i>		<i>mm.</i>	
5 to 6	56	57								
6 to 7	53	54	52	4.7	88	6.9	58	5.1	59	5.2
7 to 8	50	52	44	3.9	60	4.7	51	4.4	56	4.5
8 to 9	48	49	43	3.6	65	4.9	49	3.9	51	4.0
9 to 10	46	50	51	4.1	55	4.0	50	3.9	53	3.9
10 to 11	44	53	37	2.9	52	3.6	50	3.7	55	3.9
11 to 12	45	59	44	3.3	47	3.3	61	4.4	66	4.7
12 to 13	53	62	55	4.0	56	3.7	63	4.4	61	4.1
13 to 14	64	48	61	4.2	64	4.2	45	3.1	47	3.0
14 to 15	73	30	61	4.0	84	5.3	35	2.3	29	1.8
14.5 to 15.5			68	4.4	68	4.2	30	2.0	24	1.5
15 to 16	54	15	55	3.6	66	4.5	16	1.0	17	1.0
16 to 17	37	8	56	3.6	43	2.6	10	0.7	8	0.5
17 to 18	24	4	56	3.5	35	2.1				
18 to 19	14	1								
19 to 20	7									
20 to 21	3									
21 to 22	1									

Figures representing maximal and minimal annual growth are in italics.



TABLE II.—*Typical growth curve of the height-weight index of build. Fetal period and early infancy.*

Height.	$\frac{1}{1000}$ of height cubed.	Male.			Female.		
		Index.	Weight.	Age.	Index.	Weight.	Age.
<i>inches.</i>		<i>lbs.</i>			<i>lbs.</i>		
27	19.68	0.918	18.0	7 mo.	0.897	17.7	8 mo.
26.5	18.61	.918	17.0	6 mo.	.915	17.0	7 mo.
26	17.58	.918	16.1	5 mo.	.915	16.1	6 mo.
25.5	16.58	.918	15.2	4.5 mo.	.915	15.2	5 mo.
25	15.63	.918	14.3	4 mo.	.915	14.3	4.5 mo.
24.5	14.71	.918	13.5	3.5 mo.	.915	13.5	4 mo.
24	13.82	.918	12.7	3 mo.	.915	12.6	3.5 mo.
23.5	12.98	.918	11.9	2.5 mo.	.915	11.8	3 mo.
23	12.17	.918	11.2	2 mo.	.915	11.1	2.5 mo.
22.5	11.39	.918	10.5	1.5 mo.	.915	10.1	2 mo.
22	10.65	.918	9.8	.....	.915	9.7	1.5 mo.
21.5	9.94	.918	9.1	1 mo.	.915	9.1	.....
21	9.26	.918	8.5	2 wk.	.915	8.5	1 mo.
20.5	8.62	.918	7.9	B.	.915	7.9	2 wk.
20	8.00	.918	7.3	B.	.915	7.3	B.
19.5	7.42	.918	6.8	38 wk.	.915	6.8	.....
19	6.86	.918	6.3	36 wk.	.915	6.3	.....
18.5	6.33	.918	5.8	35 wk.	.915	5.8	.....
18	5.83	.918	5.4	34 wk.	.915	5.3	.....
17.5	5.36	.918	4.9	33 wk.	.915	4.9	.....
17	4.91	.918	4.5	32 wk.	.915	4.5	.....
16.5	4.49	.918	4.1	30 wk.	.915	4.1	.....
16	4.10	.918	3.7	28 wk.	.915	3.7	.....
15.5	3.72	.918	3.4	28 wk.	.915	3.4	.....
15	3.38	.918	3.1	27 wk.	.915	3.1	.....
14.5	3.05	.918	2.8	.....	.915	2.8	.....
14	2.74	.918	2.5	.....	.915	2.5	.....
13.5	2.46	.918	2.3	.....	.915	2.3	.....
13	2.20	.918	2.0	.....	.915	2.0	.....
12.5	1.95	.918	1.8	.....	.915	1.8	.....
12	1.73	.918	1.6	.....	.915	1.6	.....

TABLE I.—*Typical growth curve of the height-weight index of build. Early childhood.*

Height.	$\frac{1}{1000}$ of height cubed.	Male.			Female.		
		Index	Weight.	Age.	Index	Weight.	Age.
		$413 + \frac{6(X-56)^2}{1000}$			$410 + \frac{6(X-55.5)^2}{1000}$		
<i>inches.</i>			<i>lbs.</i>			<i>lbs.</i>	
38	54.87	0.607	33.3	3.5 yrs.	0.594	32.6	.....
37.5	52.73	.618	32.6	.....	.601	31.8	3.5 yrs.
37	50.65	.630	31.9	.....	.615	31.1	.....
36.5	48.63	.641	31.2	3 yrs.	.627	30.5	.....
36	46.66	.653	30.5	.....	.638	29.8	3 yrs.
35.5	44.74	.665	29.7	.....	.650	29.1	.....
35	42.88	.678	29.0	2.5 yrs.	.662	28.4	.....
34.5	41.06	.690	28.3	.....	.675	27.7	2.5 yrs.
34	39.30	.703	27.6	.....	.687	27.0	.....
33.5	37.60	.717	26.9	2 yrs.	.700	26.3	.....
33	35.94	.730	26.2	.....	.714	25.7	2 yrs.
32.5	34.33	.744	25.5	21 mo.	.727	25.0	.....
32	32.77	.759	24.9	.....	.741	24.3	21 mo.
31.5	31.26	.773	24.2	18 mo.	.756	23.6	.....
31	29.79	.788	23.5	.....	.770	22.9	18 mo.
30.5	28.37	.803	22.8	15 mo.	.785	22.3	.....
30	27.00	.819	22.1	.....	.800	21.6	15 mo.
29.5	25.67	.834	21.4	12 mo.	.816	20.9	.....
29	24.39	.850	20.7	11 mo.	.831	20.3	12 mo.
28.5	23.15	.867	20.0	10 mo.	.847	19.6	11 mo.
28	21.95	.883	19.4	9 mo.	.864	19.0	10 mo.
27.5	20.80	.900	18.7	8 mo.	.880	18.3	9 mo.
27	19.68	.918	18.0	7 mo.	.897	17.7	8 mo.
26.5	18.61	.918	17.0	6 mo.	.915	17.0	7 mo.

TABLE I.—*Typical growth curve of the height-weight index of build. Early childhood—continued.*

Height.	$\frac{1}{1000}$ of height cubed.	Male.			Female.		
		Index $413 + .6(X-56)^2$	Weight.	Age.	Index $410 + .6(X-55.5)^2$	Weight.	Age.
		1000			1000		
<i>inches.</i>			<i>lbs.</i>			<i>lbs.</i>	
50	125.0	0.435	54.4	9 yrs.	0.428	53.5	.....
49.5	121.3	.438	53.1	.....	.432	52.4	9 yrs.
49	117.6	.442	52.0	8.5 yrs.	.435	51.2	.....
48.5	114.1	.446	50.9	.....	.439	50.1	8.5 yrs.
48	110.6	.451	49.9	8 yrs.	.444	49.1	.....
47.5	107.2	.456	48.9	.....	.448	48.0	8 yrs.
47	103.8	.462	47.9	7.5 yrs.	.453	47.0	.....
46.5	100.5	.467	46.9	.....	.459	46.1	7.5 yrs.
46	97.34	.473	46.0	7 yrs.	.464	45.2	.....
45.5	94.20	.479	45.1	.....	.470	44.3	7 yrs.
45	91.13	.486	44.2	6.5 yrs.	.476	43.5	.....
44.5	88.12	.492	43.3	.....	.483	42.6	6.5 yrs.
44	85.18	.499	42.5	6 yrs.	.489	41.7	.....
43.5	82.31	.506	41.7	.....	.496	40.9	6 yrs.
43	79.51	.514	40.9	5.5 yrs.	.504	40.1	.....
42.5	76.77	.522	40.1	.....	.511	39.2	5.5 yrs.
42	74.09	.531	39.3	5 yrs.	.519	38.5	.....
41.5	71.47	.539	38.5	.....	.528	37.7	5 yrs.
41	68.92	.548	37.8	4.5 yrs.	.536	36.9	.....
40.5	66.43	.557	37.0	.....	.545	36.2	4.5 yrs.
40	64.00	.567	36.3	.....	.554	35.5	.....
39.5	61.63	.576	35.5	4 yrs.	.564	34.8	.....
39	59.32	.586	34.7	.....	.573	34.0	4 yrs.
38.5	57.07	.596	34.0	.....	.583	33.3	.....

TABLE J.—*Typical growth-curve of height-weight index of build. Later childhood, adolescence, maturity.*

Height.	$\frac{1}{1000}$ of height. cubed.	Male.			Female.			
		Index* $413 + .6(X-56)^2$	Weight.	Age.	Height.	Index $410 + .6(X-55.5)^2$	Weight.	Age.
		1000				1000		
<i>inches.</i>			<i>lbs.</i>	<i>yrs.</i>	<i>inches.</i>		<i>lbs.</i>	<i>yrs.</i>
63	250.0	<b>0.418</b>	104.5	15	63	0.444	111.0	16
62.5	244.1	.418	102.0	.....	62.5	.439	107.2	.....
62	238.3	.418	99.6	.....	62	.435	103.7	15
61.5	232.6	.418	97.2	.....	61.5	.432	100.5	.....
61	227.0	.418	94.9	.....	61	.428	97.2	.....
60.5	221.4	.418	92.5	.....	60.5	.425	94.1	14
60	216.0	.418	90.3	14	60	.422	91.2	.....
59.5	210.6	.418	88.0	.....	59.5	.420	88.5	.....
59	205.4	.418	85.9	.....	59	.417	85.7	.....
58.5	200.2	.417	83.4	.....	58.5	.415	83.1	13
58	195.1	.415	81.0	.....	58	.414	80.8	.....
57.5	190.1	.414	78.7	13	57.5	.412	78.3	.....
57	185.2	.414	76.6	.....	57	.411	76.1	.....
56.5	180.4	.413	74.5	.....	56.5	.411	74.1	.....
56	175.6	.413	72.5	.....	56	.410	72.0	12
55.5	171.0	.413	70.7	12	55.5	.410	70.1	.....
55	166.4	.414	68.9	.....	55	.410	68.2	.....
54.5	161.9	.414	67.0	.....	54.5	.411	66.5	.....
54	157.5	.415	65.4	.....	54	.411	64.7	.....
53.5	153.1	.417	63.8	11	53.5	.412	63.1	11
53	148.9	.418	62.2	.....	53	.414	61.6	.....
52.5	144.7	.420	60.8	.....	52.5	.415	60.1	10.5
52	140.6	.423	59.5	10	52	.417	58.6	.....
51.5	136.6	.425	58.1	.....	51.5	.420	57.4	10
51	132.7	.428	56.8	.....	51	.422	56.0	.....
50.5	128.8	.431	55.5	.....	50.5	.425	54.7	9.5
50	125.0	.435	54.4	9	50	.428	53.5	.....
					49.5	.432	52.4	9

\*This formula does not apply to the indices in bold-face.

TABLE J.—*Typical growth-curve of height-weight index of build. Later childhood, adolescence, maturity—continued.*

Height.	$\frac{1}{1000}$ of height cubed.	Male.			Female.			
		Index.	Weight.	Age.	Height.	Index.	Weight.	Age.
<i>inches.</i>			<i>lbs.</i>	<i>yrs.</i>	<i>inches.</i>		<i>lbs.</i>	<i>yrs.</i>
67.5	307.5	<b>0.511</b>	157.1	50+	63	<b>0.555</b>	138.8	50+
67.5	307.5	.506	155.6	45 to 49	63	.545	136.3	45 to 49
67.5	307.5	.501	154.0	40 to 44	63	.535	133.8	40 to 44
67.5	307.5	.491	151.0	35 to 39	63	.525	131.3	35 to 39
67.5	307.5	.481	147.9	30 to 34	63	.510	127.5	30 to 34
67.5	307.5	.468	143.5	25 to 29	63	.497	124.3	25 to 29
67.5	307.5	.457	140.5	20 to 24	63	.484	121.0	20 to 24
67.5	307.5	.440	135.3	19	63	.477	119.3	18 to 19
67.5	307.5	.430	132.2	18	63	.463	115.8	17
67	300.8	.427	128.4	.....	63			
66.5	294.1	.424	124.7	17	63			
66	287.5	.422	121.3	.....	63			
65.5	281.0	.420	118.0	.....	63			
65	274.6	.418	114.8	16	63			
64.5	268.3	.418	112.1	.....	63			
64	262.1	.418	109.6	.....	63			
63.5	256.0	.418	107.0	.....	63			

TABLE K.—*Relative proportions adopted as typical of various measurements during growth in stature at 5-inch interval from 20 to 75 inches, and also at 63 inches for women and 67.5 inches for men. These proportions have been made the basis of the curves in charts I, J, and K. Proportions for the female are given throughout for the stature of 63 inches, but elsewhere only where they diverge markedly from those of the male.*

Stature.	Approximate age.	Weight in pounds.	Index of build.	Volume in cubic inches.	$\sqrt{\frac{\text{Index}}{10.}}$	Approximate stature in centimeters.	Weight in kilograms.	Metric index of build, per cent.	Surface after Meeh, centim. per kilogr.
20 inches..	Birth.....	7.34	0.918	197	0.303	51	3.373	2.543	829
25 inches..	4 mos....	14.3	.918	386	.303	63.5	6.510	2.543	624
30 inches..	13.5 mos...	22.1	.819	597	.286	76	9.952	2.267	562
35 inches..	2.5 yrs....	29.0	.678	753	.260	89	13.17	1.868	462
40 inches..	4.3 yrs....	36.3	.567	980	.238	102	16.51	1.556	458
45 inches..	6.5 yrs....	44.2	.486	1193	.220	114	19.93	1.345	456
50 inches..	9 yrs....	54.4	.435	1469	.209	127	24.60	1.201	.....
55 inches..	11.7 yrs...	68.9	.414	1860	.203	140	31.39	1.144	420
60 inches..	14 yrs....	90.3	.418	2438	.204	152	40.63	1.157	421
63 inches..	20 yrs....	121	.484	3267	.220	160	54.88	1.340	.....
65 inches..	16 yrs....	114.8	.418	3100	.204	165	51.97	1.157	.....
67.5 inches..	20 yrs....	140.5	.457	3807	.214	171.5	63.81	1.265	314
70 inches..	20 yrs....	151	.441	4077	.210	178	68.85	1.221	.....
75 inches..	20 yrs....	174	.413	4698	.203	190	78.40	1.143	.....

Stature.	Approximate age.	Weight in pounds.	Index of build.	Ratio total to volume of—									
				Head to larynx.	Trunk.	Inferior extremities.	Thigh.	Leg.	Foot.	Superior extremities.	Arm.	Fore-arm.	Hand.
20 inches.	Birth....	7.34	0.918	0.280	0.495	0.135	0.063	0.048	0.024	0.090	0.0470	0.0285	0.0145
25 inches.	4 mos.	14.3	.918	.280	.495	.135	.063	.048	.024	.090	.0470	.0285	.0145
30 inches.	13.5 mos.	22.1	.819	.249	.507	.154	.071	.057	.026	.090	.0469	.0291	.0140
35 inches.	2.5 yrs.	29.0	.678	.205	.519	.186	.085	.071	.030	.090	.0468	.0292	.0140
40 inches.	4.3 yrs.	36.3	.567	.174	.522	.214	.109	.075	.030	.090	.0467	.0293	.0140
45 inches.	6.5 yrs.	44.2	.486	.149	.523	.238	.128	.080	.030	.090	.0466	.0294	.0140
50 inches.	9 yrs.	54.4	.435	.124	.518	.266	.152	.082	.032	.092	.0468	.0294	.0160
55 inches.	11.7 yrs.	68.9	.414	.104	.510	.291	.163	.088	.035	.095	.0470	.0295	.0185
60 inches.	14 yrs.	90.3	.418	.088	.512	.300	.168	.095	.037	.100	.0520	.0300	.0180
63 inches.	20 yrs.	121	.484	.072	.513	.320	.200	.093	.027	.095	.0560	.0275	.0115
65 inches.	16 yrs.	114.8	.418	.079	.528	.287	.164	.094	.029	.105	.0610	.0300	.0140
67.5 inches.	20 yrs.	140.5	.457	.074	.533	.286	.163	.094	.029	.107	.0625	.0325	.0120
70 inches.	20 yrs.	151	.441	.070	.520	.300	.175	.095	.030	.110	.0650	.0340	.0120
75 inches.	20 yrs.	174	.413	.065	.500	.323	.195	.095	.033	.112	.0650	.0350	.0120

TABLE K.—*Relative proportions adopted as typical of various measurements during growth in stature at 5-inch intervals from 20 to 75 inches, and also at 63 inches for women, and 67.5 inches for men. These proportions have been made the basis of the curves in charts I, J, and K. Proportions for the female are given throughout for the stature of 63 inches, but elsewhere only where they diverge markedly from those of the male—continued.*

Stature.	Approximate age.	Weight in pounds.	Index of build.	Ratio to stature of girth of—		Ratio to stature of transverse diameter of—						
				Head.	Chest.	Head.	Neck.	Chest.	Shoulders.	Hips.	Trochanters.	Span.
20 inches.....	Birth.....	7.34	0.918	0.688	0.670	0.200	0.110	0.215	0.240	0.170	0.210	0.950
25 inches.....	4 mos.....	14.3	.918	.670	.670	.190	.110	.215	.240	.170	.210	.950
30 inches.....	13.5 mos.....	22.1	.819	.614	.620	.170	.095	.203	.234	.174	.201	.956
35 inches.....	2.5 yrs.....	29.0	.678	.551	.571	.155	.082	.185	.227	.173	.189	.968
40 inches.....	4.3 yrs.....	36.3	.567	.501	.527	.140	.072	.174	.222	.170	.184	.978
45 inches.....	6.5 yrs.....	44.2	.486	.450	.502	.125	.068	.164	.219	.167	.179	.988
50 inches.....	9 yrs.....	54.4	.435	.412	.488	.115	.066	.157	.214	.165	.177	.998
55 inches.....	11.7 yrs.....	68.9	.414	.380	.478	.105	.063	.154	.211	.163	.176	1.008
60 inches.....	14 yrs.....	90.3	.418	.354	.475	.098	.060	.151	.210	.163	.176	1.018
63 inches.....	20 yrs.....	121	.481	.344	.481	.094	.061	.157	.213	.180	.198	1.020
65 inches.....	16 yrs.....	114.8	.418	.336	.490	.093	.062	.153	.217	.168	.181	1.028
67.5 inches.....	20 yrs.....	140.5	.467	.331	.510	.091	.063	.158	.222	.170	.187	1.040
70 inches.....	20 yrs.....	151	.441	.324	.500	.088	.062	.156	.216	.169	.185	1.037
75 inches.....	20 yrs.....	174	.413	.306	.480	.083	.060	.153	.206	.164	.182	1.033

Stature.	Approximate age.	Weight in pounds.	Index of build.	Ratio to stature of vertical measurements.								
				Head to larynx.	Head and neck.	Sitting height, male.	Sitting height, female.	Trunk to larynx, male.	Trunk to larynx, female.	Lower extremities to iliac crest.	Height trochanter, male.	Height trochanter, female.
20 inches.....	Birth.....	7.34	0.918	0.230	0.260	0.670	.....	0.440	.....	0.500	0.405	.....
25 inches.....	4 mos.....	14.3	.918	.230	.260	.670	.....	.440	.....	.500	.405	.....
30 inches.....	13.5 mos.....	22.1	.819	.225	.255	.640	.....	.415	.....	.520	.432	.....
35 inches.....	2.5 yrs.....	29.0	.678	.211	.241	.592	.....	.381	.....	.554	.460	.....
40 inches.....	4.3 yrs.....	36.3	.567	.192	.222	.567	.....	.375	.....	.576	.485	.....
45 inches.....	6.5 yrs.....	44.2	.486	.178	.208	.550	.....	.372	.....	.591	.500	.....
50 inches.....	9 yrs.....	54.4	.435	.166	.196	.535	.....	.369	.....	.605	.517	0.512
55 inches.....	11.7 yrs.....	68.9	.414	.156	.190	.524	0.527	.368	0.371	.616	.525	.518
60 inches.....	14 yrs.....	90.3	.418	.148	.188	.520	.525	.372	.377	.623	.528	.518
63 inches.....	20 yrs.....	121	.484	.143	.183	.520	.530	.377	.387	.626	.530	.512
65 inches.....	16 yrs.....	114.8	.418	.141	.186	.520	.525	.379	.384	.622	.527	.516
67.5 inches.....	20 yrs.....	140.5	.457	.138	.185	.525	.520	.387	.380	.617	.522	.....
70 inches.....	20 yrs.....	151	.441	.135	.185	.520	.....	.385	.....	.622	.525	.....
75 inches.....	20 yrs.....	174	.413	.130	.185	.505	.....	.375	.....	.630	.540	.....

Stature.	Approximate age.	Weight in pounds.	Index of build.	Ratio to stature of several measurements.							
				Knee to sole, male.	Knee to sole, female.	Height of foot.	Length of foot.	Length of upper extremities.	Length of arm.	Length of forearm.	Length of hand.
20 inches.....	Birth.....	7.34	0.918	0.220	.....	0.060	0.153	0.415	0.165	0.130	0.120
25 inches.....	4 mos.....	14.3	.918	.220	.....	.060	.153	.415	.165	.130	.120
30 inches.....	13.5 mos.....	22.1	.819	.232	.....	.059	.155	.418	.167	.133	.119
35 inches.....	2.5 yrs.....	29.0	.678	.252	.....	.057	.158	.423	.169	.137	.117
40 inches.....	4.3 yrs.....	36.3	.567	.260	.....	.055	.160	.428	.173	.141	.114
45 inches.....	6.5 yrs.....	44.2	.486	.265	.....	.054	.160	.433	.176	.144	.113
50 inches.....	9 yrs.....	54.4	.435	.270	0.272	.052	.161	.438	.180	.146	.112
55 inches.....	11.7 yrs.....	68.9	.414	.275	.277	.050	.162	.443	.184	.147	.112
60 inches.....	14 yrs.....	90.3	.418	.278	.280	.048	.163	.446	.185	.149	.112
63 inches.....	20 yrs.....	121	.484	.278	.275	.048	.150	.442	.185	.145	.112
65 inches.....	16 yrs.....	114.8	.418	.275	.277	.048	.158	.448	.189	.147	.112
67.5 inches.....	20 yrs.....	140.5	.457	.270	.280	.048	.155	.450	.190	.148	.112
70 inches.....	20 yrs.....	151	.441	.275	.....	.048	.153	.450	.190	.148	.112
75 inches.....	20 yrs.....	174	.413	.280	.....	.048	.152	.450	.190	.148	.112

TABLE L.—*Comparison of relative proportions of American college students according to variations in stature and in age.*

	Stature.		Weight.		Index of build.			
	Centi- meters.	Inches.	Kilo- grams.	Pounds.	Inch- pound.	Centim- gram.	√Cm.-Gm. Index.	
Males, after data from Hitchcock:								
Stature groups:								
a. Short.....	160	63	53.9	118.8	0.475	0.01316	0.11472	
b. Medium.....	173	68.1	62.1	136.9	.433	.01199	.10949	
c. Tall.....	183	72	68.3	150.6	.403	.01115	.10559	
c:a. Percentage plus or minus.	14.4 +		26.70 +		15.27 -		7.95 -	
Age groups:								
a. 16 years old.....	171.6	67.6	58.87	129.8	.421	.01165	.10794	
b. 25 years old.....	173.3	68.2	65.08	143.5	.452	.01250	.11180	
b:a. Percentage plus or minus.	0.99 +		10.6 +		7.39 +		3.59 +	
Females, after data from Barr:								
Stature groups:								
a. Short.....	150	59.1	45.9	101.0	.491	.01360	.11662	
b. Medium.....	160	63.0	51.4	113.3	.453	.01255	.11202	
c. Tall.....	173	68.1	59.5	131.2	.415	.01149	.10719	
c:a. Percentage plus or minus.	15.33 +		29.63 +		15.51 -		8.09 -	
	Height relative to stature.					Length relative to stature.		
	Sitting.	Supra- sternal notch.	Umbil- icus.	Pubic crest.	Right knee.	Span.	Right shoulder to elbow.	Right elbow to tip.
Males, after data from Hitchcock:								
Stature groups:								
a. Short.....	0.532	0.806	0.592	0.498	0.266	1.038	0.218	0.269
b. Medium.....	.525	.815	.601	.503	.280	1.046	.213	.269
c. Tall.....	.513	.820	.605	.500	.287	1.033	.221	.267
c:a. Percentage plus or minus.	3.75 -	1.74 +	2.20 +		7.90 +			
Age groups:								
a. 16 years old.....	.512	.825	.605	.500	.280	1.038	.218	.272
b. 25 years old.....	.527	.816	.601	.500	.272	1.028	.214	.268
b:a. Percentage plus or minus.	2.93 +	1.09 -	.06 -		2.86 -	.96 -	1.9 -	1.5 -
Females, after data from Barr:								
Stature groups:								
a. Short.....	.545	.817	.599	.490	.254	1.007	.214	.268
b. Medium.....	.532	.821	.602	.496	.257	1.016	.213	.264
c. Tall.....	.520	.818	.605	.501	.268	1.014	.213	.262
c:a. Percentage plus or minus.	4.59 -		1.00 +	2.24 +	5.51 +			2.24 -
	Breadth relative to stature.					Depth relative to stature.		
	Head.	Neck.	Should- ers.	Chest.	Tro- chanters.	Chest.	Abdomen	
Males, after data from Hitchcock:								
Stature groups:								
a. Short.....	0.094	0.065	0.258		0.196			
b. Medium.....	.089	.064	.250		.192			
c. Tall.....	.085	.060	.243		.186			
c:a. Percentage plus or minus.	9.57 -	7.69 -	5.81 -		5.10 -			
Age groups:								
a. 16 years old.....	.090	.062	.244		.186			
b. 25 years old.....	.089	.064	.257		.192			
b:a. Percentage plus or minus.	1.11 -	3.23 +	5.32 +		3.23 +			
Females, after data from Barr:								
Stature groups:								
a. Short.....	.099	.061	.229	0.158	.198	0.109	0.108	
b. Medium.....	.097	.061	.226	.157	.198	.106	.106	
c. Tall.....	.095	.059	.221	.157	.191	.1006	.103	
c:a. Percentage plus or minus.	4.04 -	3.28 -	3.49 -		3.54 -	7.71 -	4.63 -	

## HEIGHT AND WEIGHT IN RELATION TO BUILD.

TABLE L.—*Comparison of relative proportions of American college students according to variations in stature and in age—continued.*

	Girth relative to stature.										
	Head.	Neck.	Chest.	Waist.	Hips (troch.)	Right arm.	Right fore- arm.	Right wrist.	Right thigh.	Right knee.	Right calf.
Males, after data from Hitchcock:											
Stature groups:											
a. Short.....	0.350	0.210	0.532	0.439	0.548	0.155	0.158	0.101	0.313	0.213	0.203
b. Medium.....	.331	.205	.507	.420	.517	.150	.152	.097	.301	.209	.203
c. Tall.....	.318	.195	.491	.409	.504	.144	.147	.094	.289	.201	.195
c:a. Percentage plus or minus.....	9.14—	7.14—	7.71—	6.86—	8.03—	7.16—	7.02—	6.93—	7.67—	5.63—	3.84—
Age groups:											
a. 16 years old.....	.328	.200	.500	.411	.498	.146	.147	.096	.292	.208	.199
b. 25 years old.....	.330	.212	.529	.444	.528	.153	.155	.097	.304	.207	.205
b:a. Percentage plus or minus.....	.61+	6.00+	5.80+	8.03+	6.00+	4.79+	5.44+	.....	4.11+	.....	3.02+
Females, after data from Barr:											
Stature groups:											
a. Short.....	.357	.197	.490	.393	.547	.145	.135	.093	.337	.217	.202
b. Medium.....	.343	.189	.479	.390	.561	.143	.143	.094	.344	.216	.208
c. Tall.....	.328	.182	.470	.403	.551	.146	.142	.094	.353	.218	.211
c:a. Percentage plus or minus.....	8.12—	7.61—	4.08—	2.54+	.....	.....	5.11+	.....	4.75+	.....	4.46+

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